Hardy Fern Foundation Quarterly

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Special Publication
on Propagation

Editor Sue Olsen Volume 8 Number 2

THE HARDY FERN FOUNDATION

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The Hardy Fern Foundation was founded in 1989 to establish a comprehensive collection of the world's hardy ferns for display, testing, evaluation, public education and introduction to the gardening and horticultural community. Many rare and unusual species, hybrids and varieties are being propagated from spores and tested in selected environments for their different degrees of hardiness and ornamental garden value.

The primary fern display and test garden is located at, and in conjunction with, The Rhododendron Species Botanical Garden at the Weyerhaeuser Corporate Headquarters, in Federal Way, Washington.

Satellite fern gardens are at the Stephen Austin Arboretum, Nacogdoches, Texas, Birmingham Botanical Gardens, Birmingham, Alabama, California State University at Sacramento, Sacramento, California, Dallas Arboretum, Dallas, Texas, Denver Botanic Gardens. Denver, Colorado, Georgeson Botanical Garden, University of Alaska, Fairbanks, Alaska, Harry P. Leu Garden, Orlando, Florida, Coastal Maine Botanical Garden, Wiscasset, Maine, Inniswood Metro Gardens, Columbus, Ohio, New York Botanical Garden, Bronx, New York, and Strybing Arboretum, San Francisco, California.

The fern display gardens are at Lakewold, Tacoma, Washington, Les Jardins de Metis, Quebec, Canada and University of Northern Colorado, Greeley, Colorado.

Hardy Fern Foundation members participate in a spore exchange, receive a quarterly newsletter and have first access to ferns as they are ready for distribution.

Cover Design by Willanna Bradner.

SPECIAL ISSUE-

PROPAGATION

Introduction

Growing ferns whether as the primary plants in the garden or as companions is an increasingly popular hobby. For many of us this eventually leads to an interest in propagation. The Hardy Fern Foundation has received many inquiries requesting information on growing methods so we asked our readers to contribute descriptions of their programs for this special issue. I have always believed that there are as many variations in propagating methods as there are growers. This indeed turned out to be the case with the articles we received as well as in the concluding propagation discussion reprinted from the Pteridonet (now Fernet).

Joan Gottlieb gets us started with a description of the essentials of the life cycle of ferns as well as with an account of apogamy and apospory.

Martin Grantham presents some observations on reproduction in xeric ferns in an article extracted from a longer manuscript to be published later on the xeric ferns at the UC Berkeley Botanical Garden.

From there we get into the particulars of growing ferns from spores which is the primary focus of this issue. We begin with the basics of collecting and cleaning spores. Alastair Wardlaw follows with a comprehensive account of both his methods and results. Bob Muller, James Horrocks and Sue Mandeville contribute further ideas all using organic mediums. Unusual alternatives are presented by Jim Baggett, Peter Podaras and Samuel Tumey the latter article being a reprint from the Hardy Fern Foundation Newsletter Volume 5 Number 2 of Spring 1995. Finally Martin Rickard gives an account of vegetative propagation and Serge Zimberoff discusses tissue culture.

While I did not present a description of my methods, I would like to comment regarding two areas where I differ from the other systems. First we use an organic earth worm compost mix for sowing the spores. This is moistened put in a handy Pyrex lasagna pan, covered with foil and baked at 175 degrees for three hours. (Be advised it is fragrant!) The low temperature rids the soil of contaminants but does not cook away the "good" ingredients as would happen with a higher temperature. Also I do not use distilled water as I've been told that distilled water lacks the necessary minerals for healthy growth. I do let the water spray container sit for 24 hours after filling, however, to let the chlorine evaporate.

An article on hybridization is being prepared by Herb Wagner and will be published in a later issue. This will add to the challenge and excitment of exploring and creating interesting new types through propagation by spores.

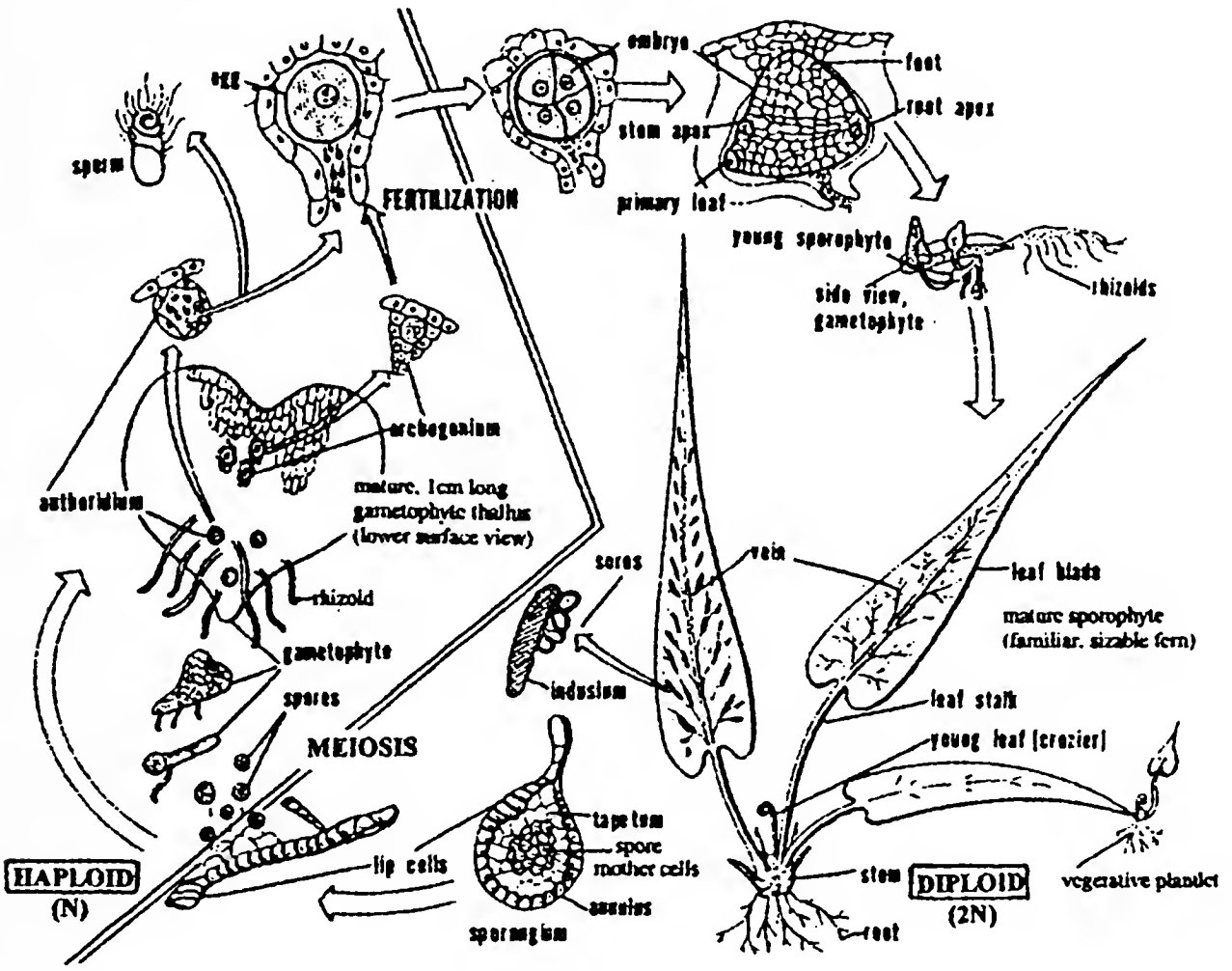
Sue Olsen

The Sex Life (or Life Cycle) of Ferns

Joan Eiger Gottlieb - Pittsburgh, PA

If the title has grabbed your attention, be assured that ferns, like all plants, do indeed have an active, exciting sex life. But, it does not take place on the plant body that is familiar to us. The feathery-leafed *Polystichum*, with its fuzzy fiddleheads, forest-green fronds, creeping or crown-forming stems, and ground-hugging roots is called the **sporophyte** (spore-producing plant) and it has no sex life! If we let "N" represent a complete set of chromosomes for a species, then the body cells of the sporophyte are usually 2N (**diploid,**) having two complete sets of chromosomes, inherited from typically **haploid** (N) parental egg and sperm cells – each contributing one set.

LIFE CYCLE OF THE WALKING FERN (*Camptosorus rhizophyllus*)



So, the *Polystichum* in your garden starts out as a fertilized egg (a 2N zygote) which grows by mitosis/cell division into a ball-shaped embryo. Cells at one end enlarge and differentiate into a root and, at the other end they form a shoot (a stem and juvenile leaf). Both root and stem retain a permanently embryonic cell (or cells) termed a meristem which continues to produce new cells and highly specialized tissues like xylem and phloem (for support and conduction,) enabling the baby sporophyte or sporeling to enlarge, branch, and produce successively larger, more elaborate leaves. Growth to maturity may take a few months or a few years, depending on the species and the growing conditions. At maturity, special cells of some fronds develop into protruding sacs called sporangia. Each sporangium has a protective jacket of thick-walled cells (the annulus) and contains a mass of 16 spore mother

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The Sex Life (or Life Cycle) of Ferns *continued from page 1*

cells surrounded by a nutritive tapetum. Sporangia are usually formed in groups called sori (sing. = sorus) or "fruit dots" on the abaxial (under-) side of fertile fronds, but other leaf/sorus arrangements are common. In *Acrostichum* the entire abaxial leaf surface is covered with sporangial masses; in *Polypodium* round sori are confined to the tips of each fertile frond. And these are only a couple of variations on this theme. The type, shape, and arrangement of sori, the presence of protective leaf outgrowths (indusia) vs. recurved leaf margins, etc. are important in fern taxonomy.

The typically 2N spore mother cells (s.m.c.) inside each sporangium undergo a special double nuclear division called **meiosis** whereby the two original sets of parental chromosomes are "shuffled" and their number is halved. The four spores resulting from each meiosis are haploid (N) and are thus quantitatively different from their 2N mother cell. They are also qualitatively distinct (uniquely re-arranged genetically) from the original egg and sperm that formed the sporophyte plant, thus enhancing variation. This meiosis is the same process that occurs in the ovary or testis of animals, but in plants the resulting daughter cells are not eggs and sperms. They are non-sexual reproductive cells – the dust-like **spores** that are catapulted from ripe sporangia and drift to the ground. Some ferns and fern allies (e.g. *Osmunda, Equisetum*) produce green spores which are short-lived and must germinate quickly or perish. Other ferns have dark spores with thick, ornate, species-distinctive spore walls that can survive for many months or years. With each sporangium making 64 spores, each sorus having 50+/- sporangia, and each fertile leaf bearing dozens or even hundreds of sori, prodigious numbers of spores can be shed each season by a single, mature sporophyte. However, only a small percentage of fern spores land in places suitable for their germination.

In any case the haploid spore is the start of something brand new – a **gametophyte** (gamete-bearing plant). It begins life on a moist, shaded substrate (soil, potting mix or various culture media) as a tiny, green filament (string of cells) emerging from the spore wall by mitotic division and cell elongation. Some ferns (e.g. *Trichomanes*) retain a filamentous gametophyte form, but in most ferns, perpendicular divisions soon build a two-dimensional strap- or ribbon-shaped gametophyte. This simply elongates and branches to maturity in some species (e.g. *Hymenophyllum*). In most ferns the small ribbon soon broadens into a heart shape with a permanent, growth meristem in its "notch" and hair-like rhizoids on its lower side for anchorage and absorption. This flat, tissue paper-thin, simple plant, in all its forms, is called a prothallus, and it may be the vestigial expression of an ancient green algal ancestor, which it resembles quite remarkably. The fully formed prothallus is one cell thick at its margins and only a few cells thick near its central "cushion" behind the meristem. In nature it is a "prayer bones" find, for it grows over a period of weeks or months only to the size of a pinky nail – hard to spot against dark, forest soils. Look for prothalli under mature ferns, on clay soils near streams, in damp rock crevices, or on undisturbed pots in humid greenhouses. Underside cells of the prothallus produce antheridia (sacs containing a few sperms) among the rhizoids and archegonia (vase-like containers, each with a basal egg) near the meristem. These reproductive organs are formed by mitosis, so all are typically haploid like the gametophyte itself.

And, in bisexual ferns, they are produced in successive waves, increasing the chance for cross-fertilization. Some ferns have unisexual gametophytes, bearing either antheridia or archegonia, but not both, thus ensuring and maximizing the advantages of outcrossing. There are ferns and fern allies with underground, three-dimensional, axial (cylindrical) or carrot-shaped gametophytes (*Botrychium, Psilotum, Lycopodium*) believed by some botanists (D. Bierhorst et al.) to be a primitive type.

Following a rainfall, large, spiral-shaped, multi-flagellated sperms are released from the opening "caps" of the antheridia and swim down narrow, archegonial necks to fertilize the eggs. They are guided by attractants like malic acid emanating from the neck cells. Usually, only one egg is fertilized per gametophyte because the first zygote to form inhibits adjacent archegonia. Early divisions of the zygote (the 2N cell that is the official start of the sporophyte generation) establish an absorbing "foot" at the base of the archegonium enabling the enlarging embryo to draw nutrients from its parent gametophyte. This dependent baby sporophyte soon kills the gametophyte that produced and nourished it. Enlarging and successively more complex leaves shade the parental prothallus, and hormones flowing from the sprorophyte's growth tips inhibit the delicate, but sexy plant, which simply withers away at the base of its burgeoning, newly independent offspring. The gametophyte stage, while essential for sex and the genetic recombination it offers, is thus reduced in ferns to a tiny, ephemeral, but still free-living plant with risky, water-dependent fertilization. It can be viewed as a transitional, two-dimensional stage for the sexual drama that creates a larger, three-dimensional, long-lived, complex fern sporophyte – the truly land-adapted, but, alas, non-sexual plant!

Much controversy exists over how the plant life cycle with its remarkable alternation of gamete-bearing and spore-bearing stages got started. Chromosome numbers alone cannot account for the striking differences between typical fern gametophytes and sporophytes. Gametophytes are known with 2N, 3N, 4N and even larger (polyploid) chromosome sets. They still grow flat and have fertile gametes in most cases. The sporophytes they produce are normal in appearance, and, if they have even chromosome multiples (4N, 6N, etc.,) they can usually undergo meiosis and make viable spores. A surprising amount of fern evolution may, in fact, be based on such spontaneous polyploidy. Accumulated, experimental evidence points to environmental influences such as nutrition, O_2/CO_2 balance, photoperiod, light wavelength, physical pressure, and chemical gradients on the genome selections and developmental pathways that shape a gametophyte from a spore or a sporophyte from a zygote. For example, fertilized eggs removed from archegonia and placed on a nutrient medium in tissue culture grow into somewhat flatter, less complex masses. However it came about, in ferns it is the complex, spore-producing plant that is better adapted for life on land. And the sporophyte typically has at least a double set of chromosomes to withstand the mutational hazards of that life.

There are important variations on this Jekyll and Hyde life cycle among Pteridophyta (the umbrella group or phylum to which ferns have been assigned.) After all, this is a large assemblage of ancient, diverse, and often obscurely-related members. Consider, for example, the "primitive" Whisk "fern" (*Psilotum,*) which has subterranean, fungus-associated gametophytes, indistinguishable from its sporophyte rhizomes except for their production of gametes (rather than the upright, green fronds or

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The Sex Life (or Life Cycle) of Ferns ***continued from page 3***

"aerial shoots" of the latter.) Then there is the Clover fern (*Marsilea*) and related aquatic genera with submerged sporocarps (specialized, toughened leaf bases containing large and small spores (**heterospory**) producing female and male gametophytes, respectively. In horsetails (*Equisetum*) and most club mosses (*Lycopodium*) the spore-bearing leaves are clustered together, forming cones. Spike "mosses" (*Selaginella*) have heterosporous cones, with sporangia containing large spores at the base of the cone and sporangia with more numerous, small spores at the top. There is even a type of "pollination" in these heterosporous fern allies with delayed release of a well-developed, young sporophyte from the protective cone. These sidebars to the **homosporous** (all spores the same small size) life cycle of typical ferns appear to have been evolutionary dead ends. They are not on the same line as the heterospory which arose in a group of Paleozoic "seed ferns" and led to the true seed plants (Spermatophyta.) But that, as they say, is another story!

Apogamy and Apospory for Amateurs

Joan Eiger Gottlieb - Pittsburgh, PA

The life cycle of "typical" ferns has been discussed elsewhere in this newsletter. You might say this article is "the rest of the story." It turns out that, in addition to producing special, reproductive cells (eggs, sperms, spores) on alternating sexual and asexual plants (gametophytes and sporophytes, respectively,) most ferns increase their numbers through a variety of vegetative techniques. These do not involve the genetic recombination that takes place during fertilization and meiosis; they simply clone the parent plant, if you will. For example, sporophytes often increase their populations by forming detachable buds, stolons, bulbils, and plantlets. Gametophytes duplicate themselves by shedding simple gemmae (small masses of surface cells) or by growing many lobes that break apart as older parts of the thallus wither. These special structures and growth patterns can be very advantageous in spreading fern populations locally during rain-scarce periods, since they effectively multiply the result of fortuitous sexual unions or spore sproutings.

But, the most intriguing of the vegetative strategies used by ferns are **apogamy** and **apospory**. Apogamy literally means "without gametes" and it refers to the production of a sporophyte directly from a body (somatic) cell of a gametophyte (rather than from an egg or a zygote.) The body cell doing the job may be part of the archegonial jacket; it may be from an abnormal antheridium; or it may be from the thickened "cushion" of cells near the meristem. A young, apogamously-produced sporophyte is, initially, a small mass of cells multiplying rapidly and resembling an embryo. But, the absorbing foot that always connects a sexually-produced embryo to its parent gametophyte is missing; and, most tellingly, the cells of the apogamous mass have the same (typically haploid) chromosome number as the gametophyte. A visual clue that an apogamous sporophyte is developing is the appearance of dense masses of multicellular hairs on the surface of the gametophyte thallus in the area of the swelling mass. Another indication of apogamy is the appearance first of a leaf (sexually produced embryos put out a root first). Apogamy is enhanced under high light intensities in nature or extra sugar in laboratory cultures. It is probably common in ferns when fertilization fails to occur or when there are

chromosome anomalies. For example, desert ferns (*Pellaea*, *Cheilanthes*) and hybrids (*Dryopteris remota*) are frequently apogamous. D. Whittier's work (Phytomorphology, March, 1962) suggests that conditions favoring high nutritional (energy) states in the 2-dimensional gametophyte thallus may shift cell development to the more complex, 3-dimensional sporophyte form.

Apogamous sporophytes have normal-tissued roots, stems and leaves, and often grow to adult size, producing viable spores at maturity. *Dryopteris affinis*, the Golden-scaled male fern of Europe and Asia, is an entirely apogamous species. Its gametophytes have imperfect sex organs, but they regularly produce, by apogamy, the large, elegant, lustrous-leaved ferns and the many cultivars of this species that are


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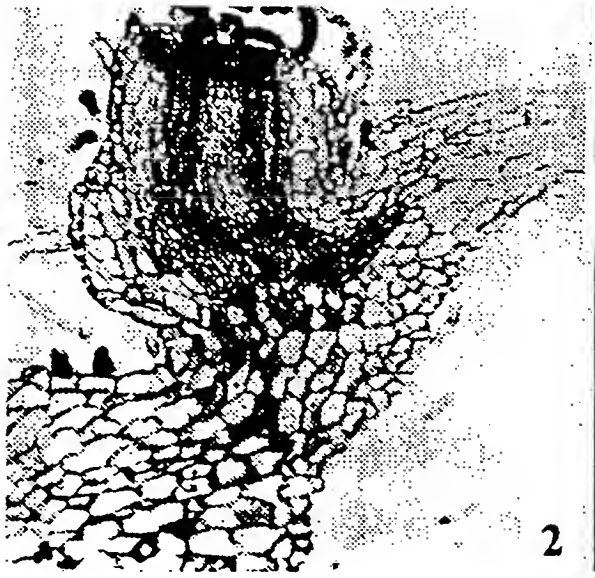
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Apogamy in *Pteridium* (Bracken Fern)

1) Group of apogamous plants arising from thickened center of prothallus. Arrows point to shoot meristems (growth tips).
2) Section of an apogamous "bud" with prothallus at its base.
3) Isolated apogamous leaf; note fairly advanced, pinnate structure.

(From D.P. Whittier "The Origin and Development of Apogamous Structures..." Phytomorphology 12(1): March, 1962.)

so popular in American gardens. The spores of *D. affinis* are formed by ordinary cell division, without the genetic sorting of meiosis so the resulting prothalli are all identical – true clones! Diploid gametophytes (*Psilotum*, *Vittaria*, among others) produce diploid sporophytes apogamously, so the ability to multiply this way is not chromosome limited.

On the sporophyte side of the life cycle is the counterpart process of apospory – literally "without spores." In this case sporophyte plants generate entirely normal gametophytes directly from cells on the surface of their leaves, without the intermediary of meiosis and spore formation. Apospory occurs in nature when juvenile leaves of young sporophytes are detached and lie prostrate on damp soil. It is easily induced *in vitro* by culturing small leaves or individual pinnae on appropriate, low carbohydrate growth media. Under such reduced nutritional conditions, proliferous growths soon form from epidermal cells along the leaf margins. These may remain as unorganized masses; they may grow out as sporophyte buds; or, they may become lobular, functional gametophytes. Since leaf tissue is typically

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Apogamy and Apospory for Amateurs *continued from page 5*

diploid (2N,) these aposporous gametophytes will also be 2N as will their sex organs and gametes (eggs and sperms.) Fertilization will give rise to tetraploid (4N) babies, an instantaneous doubling of the species number – sometimes of evolutionary significance in the formation of new species. And so, the chromosomal chaos continues!

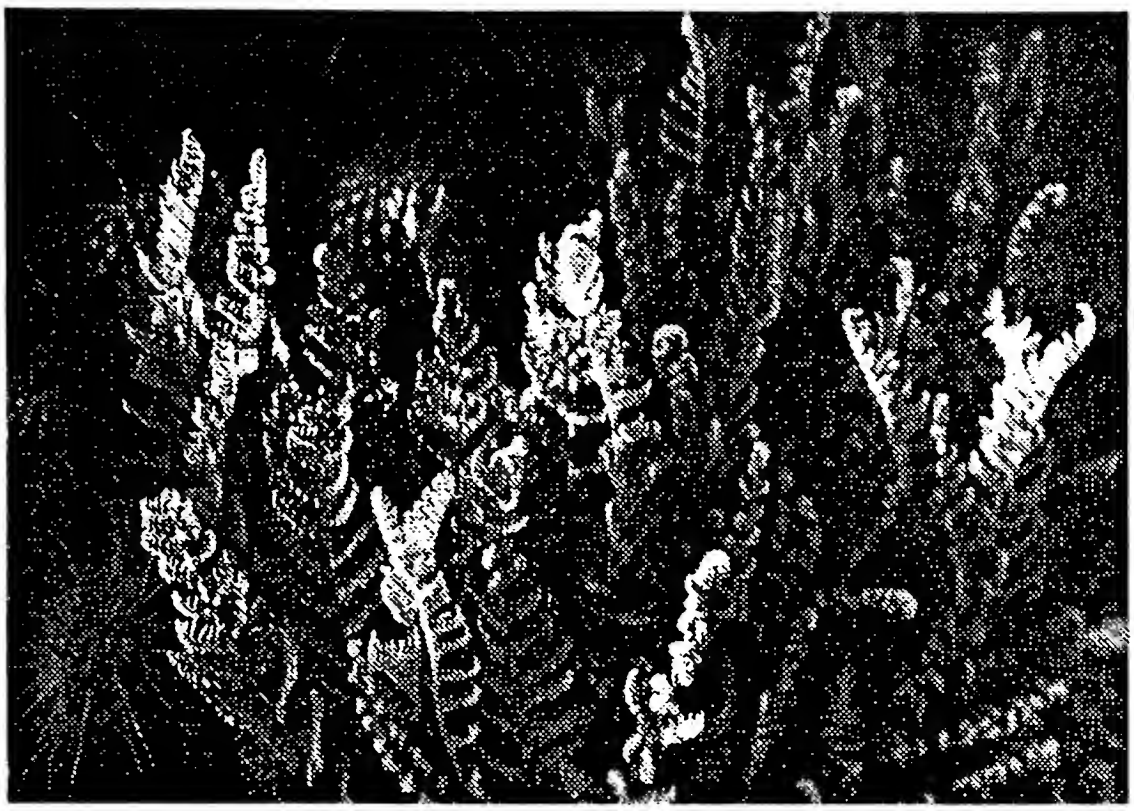
What sense is to be made of this chromosomal promiscuity in ferns? (It occurs in seed plants as well.) We do not know what induces a particular cell to express the gametophyte or sporophyte pattern as it divides and grows. Chromosome number, however valuable taxonomically, clearly plays only a small and easily overriden part.

Nutritional status, position on the plant, and attachment effects are similarly incomplete explanations. There is a wide range of cell, tissue, organ, and morphological "expression" that characterizes ferns. A certain "set" of these is seen in the typical gametophyte thallus and another set occurs in the average sporophyte plant, but there is considerable, if not immediately apparent overlap. Tracheids (the water conducting cells of the sporophyte) can be found in some older gametophytes and flat, thallus-lobes can form on sporophyte leaves. There are striking similarities in the initials (beginning cells) of antheridia, archegonia and sporangia. The young hairs and scales of gametophytes and sporophytes are incredibly similar. Subsequent differentiation and specialization are what distinguish them. It is like having computer software with several applications. The user has the ability to access individual applications or to integrate parts of them in new programs. Back in 1936 A.J. Eames postulated that gametophytes and sporophytes are fundamentally alike and represent "correlative phases in the life cycle...which arose by modification of an ancestral (algal) phase which was sexual." This is the developmental sameness or **"homologous"** theory. So, the gametophyte and sporophyte, however different they may seem in their mature form, may really be malleable expressions of a common potential that is influenced and balanced by environmental conditions, ergo apogamy and aposporγ. In the case of a zygote, containment and attachment at the base of the archegonium may exert the directional and nutritional influences that select the sporophyte morphology from the total plant genome. The free-living spore has a very different developmental milieu, selecting a growth pattern that is flatter and simpler – the gametophyte body type. And that, whether we can explain it completely or not, is the way plants do it!

Vocabulary addendum for the word-addicted: **Apomixis**, a word frequently encountered in the literature, means the substitution of an asexual process for the normal sexual union of egg and sperm. It includes parthenogensis (the stimulated development of an embryo from an unfertilized egg) as well as apogamy (where the development is from a body cell.) Both processes occur in plants on the gametophyte thallus. Apomicts (the plants resulting from apomixis) are usually haploid; but, like the parent plant that forms them, they can be 2N, 3N, 4N, etc.

Reproduction of Xerophytic Ferns*

Martin Grantham - UC Botanical Garden - Berkeley, CA



At U.C. Botanical Garden, Berkeley. *Notholaena buchtienii.* Photo by Sue Olsen

Reproduction by spores would hardly seem an advantage in dry regions. Yet this might in fact make xerophytic ferns better than flowering plants at finding the restricted microhabitats in otherwise dry terrain that provide enough water for establishment and completion of the life cycle. Spores are released in far greater numbers than the seeds of flowering plants and, because of their small size, spores are much more widely dispersed. If a particular rock formation is arranged in such a way that precipitation (rain or dew) is harvested and funneled to a favorable spot, it won't be long before these same rocks are insulating the roots and rhizomes of ferns from midday heat. (Rocks have been shown to provide a temperature reduction of up to 15 degrees F in the field.)

The spores produced by ferns from dry habitats tend to be deeply pigmented with phytomelanin, a pigment closely related to the melanin which protects our own skin from UV damage. They are long-lived in storage and probably relatively long-lived in nature. (For ferns in general there is a broad range of 'shelf life' from a matter of days in 'green spored' *Osmunda* to under a year for *Cyathea* up to a record of 70 years for *Plagiogyria*.) Although xerophytic fern spores may be exceptionally tough, the life cycle stage to emerge from these spores, the gametophtye or prothallus, is very delicate and tiny. It consists of a central pad with several cell layers and two lateral wings a single cell layer thick. At the growing tip there is a notch which gives the entire structure the shape of a heart. The life cycle role of the gametophtye, as

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*This is an excerpt from a longer article - Xerophytic Ferns at UC Berkeley Botanical Garden to be published at a later date.

Reproduction of Xerophytic Ferns *continued from page 7*

the name indicates, is to produce gametes. The male gamete is motile and requires a continuous film of water in order to reach the sessile female gamete. Fertilization results in the development of the sporophyte (so named because it eventually produces the spores through meiosis.) The sporophyte is the stage that comes to mind for all of us when we think of a fern.

REPRODUCTIVE QUIRKS

At this point you may be asking yourself: How can these delicate water-dependent life cycle stages and events occur under xeric conditions? Basically the answer is speed via accelerated development or the elimination of certain steps. Alternatively, some fern gametophytes, as in Pellaea, can enter a state of physiological "suspended animation" during dry periods. The only structural adaptation to dryer habitats I have noticed in the gametophtyes of xerophytic ferns is the production of wax (*Notholaena, Argyrochosma* and *Pellaea*) which may reduce water loss.

Accelerated development may allow all vulnerable life cycle stages to occur during a relatively brief and perhaps rare damp period. Among ferns in general there is a broad range for the minimum time required to progress from spore to sporophyte. Xerophytic ferns are among the fastest, producing sporophytes in as little as 6 weeks in my experience while slower ferns may require two years or more. To save time many xerophytic ferns simply skip fertilization. An embryo forms directly from the gametophyte's central pad of tissue. This is called "apogamy" which means "without gametes". Abnormalities in meiosis resulting in diploid spores makes this type of development possible. In *Pellaea* gametophytes may have the ability to survive long periods of extreme desiccation during which they are quiescent. This would allow development to proceed cumulatively over sporadic wet periods.

Getting Started - Gathering and Cleaning Spore

Sue Olsen - Bellevue, WA

Fern enthusiasts have numerous sources for obtaining spores. The Hardy Fern Foundation, American Fern Society and British Pteridological Society all have spore exchanges with contributions from around the world. Friends and fellow enthusiasts and correspondents often share spores and, of course, you can always collect your own (and make the exchange curators happy by donating the extras as well.)

Collecting spores takes a little practice (and some careful observation) but is not difficult if you follow these guidelines. Spores are normally on the underside of the frond In compartments (cases) known as sporangia which in turn are gathered into clusters, known as sori. These are often covered by a thin membrane called the

indusium. The shape of this indusium varies by genus and is one of the determining factors in the identification and classification of ferns. Immature indusium covering unripe spores is usually a pale creamy green and firmly attached to the frond. Generally the spores are black or brown in color and as they ripen they lift the indusium and prepare for flight. A hand lens helps in following the process. To test for ripeness pick a small pinnae and place it spore side down on smooth white paper. The spores should drop within 24 hours and will be rounded globes. Quite frequently there will also be copious quantities of irregularly shaped debris - the chaff. If spore does drop you're ready for action. Otherwise patience please! The spores ripen from the base of the frond upwards toward the tip so there is some latitude in timing. In our garden spores begin to ripen in late May usually with the *Polystichums* and continue throughout the summer with the *Adiantums* bringing collecting to a close in late September. Light brown ragged and shriveled indusium indicates that the spores have been dispersed and it is too late to collect.

Not all species have an indusium to prolong the distribution of spores and not all species carry the spores on the underside of the frond. *Osmundas* bear spores on specialized sections of the fronds. Their spore is green when ripe which is usually early May. To test for ripeness gently tap the sporangia. When ripe the spores will waft away like a small cloud of green smoke and should be collected immediately. They are only viable for a short time and should be sown at once but can be refrigerated for up to two weeks.

Among others the *Polypodiums* also are without an indusium and the spores essentially ripen all at once. They are, however on the underside of the fronds and frequently a brilliant yellow. They need to be closely watched for an appropriate harvesting time.

Cleaning the spores is in my opinion the most important element in encouraging successful propagation. When a fertile frond is picked it is a good idea to lightly rinse it in water to remove any rogue spores that may have floated in from nearby ferns. (This is especially true if there are any friendly *Athyrium filix-femina* in the area.)

Meanwhile, It is quite likely that the spore packet will contain a mixture of spores and chaff. Chaff is highly likely to contaminate a culture and needs to be canted off. The easiest way is to take the paper with your collected material tip it slightly and then gently tap. The chaff will roll off leaving a dust like residue of spores. This procedure can be quite alarming for the neophyte as it often looks as if the whole deposit is falling away (and if you've received a packet with incorrectly collected spores this may actually be the case!!!) To be absolutely certain that the chaff has been removed some growers sift the spores through camera lens cleaning tissue. A good way to learn is to practice with a common local fern from which multiple collections can be made.

Now all you need to do is to choose a growing method and be aware that propagating can become an incredibly rewarding addiction!

Waiting for Sporophytes

Alastair C. Wardlaw - Glasgow, Scotland

The Editor of this Newsletter persuaded me that readers might be interested in seeing the records I have kept for the lengths of time taken for spores to germinate and give prothalli and for the subsequent development of sporophytes. I have therefore summarized this information with 109 spore samples from the last 4 years and I offer it (Table) for what it is worth. I suspect it may not be worth very much from the standpoint of making generalizations, because there are so many variables that affect growth rate. But perhaps it may provoke some discussion. I do not recall seeing a similar tabulation elsewhere and I shall certainly be interested in getting feedback. In summary, the average time for appearance of developing prothalli, visible through a 10x hand lens, was 32 days and the median time for appearance of sporophytes was 106 days, but read on.

Choice of spores

I have been growing ferns in my garden in Glasgow, Scotland, since 1970 when we moved here from Canada. But only during the last 5 years have I made extensive efforts with spore cultures. Prior to that, most of my ferns were bought as grown plants from specialist British nurseries and some of the common ones were collected from the wild. At present, I have about 400 different species and varieties of fern.

Initially I was interested only in British ferns and their numerous cultivars, mainly because I did not realize that many species of alien fern would also grow well in a Scottish garden. Subsequently, I have been collecting ferns from other temperate lands, notably Continental Europe, North America, the Himalayas, China, Japan and Korea, South Africa, and Australia and New Zealand. The spore samples listed in Col. 1 of the Table were thus selected to supplement an already substantial collection of ferns, of types commonly available from suppliers and previously grown from spores.

Prior to 1995 I had grown about 80 spore cultures but without keeping records of the times to produce prothalli and then sporophytes. The 109 spore samples in this Report are subsequent to these initial experiences. The methods described below are what I now use and recommend.

Sources of spores

The annual spore lists of the British Pteridological Society (BPS) for 1995-97 and the American Fern Society (AFS) for 1997 provided 58 of the samples shown in the Table. The remainder were sent to me by private donors (PD) or were collected during a 1996 trip to New Zealand (NZ), with a few from Australia included under 'NZ'. Col. 2 of the Table sets out the particulars of the sources.

Age of spores

This may be relevant since spores presumably do not remain viable indefinitely. Col. 3 shows that the ages of the spores ranged from 3 months (expressed as 0.25 year) to 5 years. The samples collected in New Zealand and those sent by private donors were all less than one year old when I sowed them. The spore samples from the fern societies were mainly no more than one or two years old. Note that the ages are only approximate since a fern-society sample from the same calendar year as the society spore list is tabulated as having an age of 0.5 year. There did not seem to be any obvious association between age of spore and time to prothalli.

Growth medium

Spores are sown onto a medium consisting of equal volumes of: moss peat, a rather clayey garden soil (with twigs and stones removed) and 6-mm granite chips. A trace of bonemeal is sometimes added. In the future I shall probably incorporate some vermiculite. The well-moistened growth medium is filled into ordinary 8-cm diameter plastic plant pots with about a 1 cm depth of 12-mm granite chips in the base for drainage. Each pot is put into a transparent freezer bag and sterilized (4 pots at a time) by heating to about 90-95°C in an 800-watt microwave oven. With each batch of pots, the time for microwaving (generally 4 min at medium power) is found by pushing a thermometer into the soil in the center of a pot immediately after heating for a trial period. It is important not to overdo the microwaving as the temperature can easily rise to above 100°C and melt the pot and the plastic bag.

Growing conditions

The cooled spore pots, with their surfaces freshly moistened, are inoculated from the spore-sample packets as received and without any attempt to sterilize the spores. However precautions are taken to minimize cross-contamination during sowing. Each plastic bag, after spore sowing, is closed with a twist tie. The inoculated pots are then put into a homemade plexiglass box (100 x 32 x 31 cm), with lid and front wall detachable for access. The soil surface is about 35 cm below twin 30-watt Gro-Lux (Sylvania) fluorescent lights, with a reflector. The box is located in a West window and the outside long wall and sides are covered with aluminium foil to prevent sun scorching of the interior. A light meter shows that an average of about 1400 lux (range 1200-1800) is delivered by the fluorescent tubes at the level of the soil; this intensity being reduced by the plastic of the bags by about 10%. This supplied light intensity is equivalent to about 3% of summer midday sunlight (in Glasgow). The lights are on an 18 hour/day cycle, and the pots also receive some indirect daylight through the window, with periods of up to 3000 lux when the sun is shining. The temperature inside the box is measured with a min/max thermometer, but not controlled, and varies with the season and the time of day. It ranges from 14 up to about 26°C, the average being around 20°C.

Observations of growth

At irregular intervals of about 5-8 days, each pot is taken out of its plastic bag and inspected through a 10x hand lens. If the surface has no visible moisture then a few squirts of tap water are gently applied. The date on which developing prothalli became convincingly visible through the hand lens is recorded; subsequently the date of appearance of sporophytes is noted. Problems of fungal or algal contamination are also recorded if serious. If prothalli grow to give a confluent covering of the soil surface they are thinned out with forceps. Mosses are also kept in check if required, by forceps weeding. The few prothalli cultures that become overgrown with algal slime, or which have not yielded sporophytes after about 6 months are transferred as well-spaced clumps onto fresh, sterile growth medium and incubation continued.

When sporophytes are about 1.5-4 cm high, they are transferred to individual peat pots and subsequently to 8-cm pots of the same microwave-sterilized medium as used for the spore cultures. Calcicole plants have lime added at the second transplantation. One-and-a-half to two years, sometimes longer, are generally required to produce a fern of garden-ready size from the initial sowing of spores.

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Waiting for Sporophytes *continued from page 11*

Spore pots after harvest of sporophytes are not necessarily discarded but are kept in their closed plastic bags and put into 'dead storage' in a shady part of the garden. They and then inspected occasionally for up to several years to see if anything further has grown.

Results

Prothalli: Column 4 of the Table contains the times I recorded when developing (obviously still very immature) prothalli were first seen under the hand lens. Because of the intermittent nature of the inspection process at 5-8 day intervals, the times recorded for the observations are accurate to within a day, but the relevant growth events could have occurred a few days earlier, especially for the longer times. So that in Col. 3, for example, recorded times of 13 and 17 days for prothalli appearance may not reflect a genuine difference between spore samples but be due to the different time intervals between inspections for different batches of pots. However, despite this limitation, there did appear to be genuine differences in the times taken for prothalli to appear. The shortest recorded time was 11 days and the longest was 69. With several of the species (*Adiantum reniforme, Athyrium thelypteroides, Cheilanthes ineaqualis, Dryopteris dracomontana, D. arguta and Thelypteris hexagonoptera*), spore samples from more than one source or locality were tested, with somewhat variable results. The overall average recorded time for the first appearance of developing prothalli in the 109 spore samples was 32 days.

Prothalli-free culture pots were generally kept in the growth chamber for up to about 4-6 months (120-180 days) before being recorded as 'sterile' and put into dead storage. Not shown in the Table are 13 samples from the fern societies' lists that failed to yield prothalli or which became overgrown with algae, fungi or mosses and could not be rescued. Possibly, some of these failures could have been prevented by sterilizing the spores before sowing, or by using a different growth medium.

Sporophytes: The times for appearance of sporophytes (Col. 5) were much more variable than for prothalli, some being longer than one year. The shortest recorded time was 41 days for *Cosentinia vellea*, a Mediterranean dry-rock species. Other species that yielded sporophytes in 60 or fewer days were *Adiantum reniforme, Cheilanthes hirta, Cyrtomium lonchitoides, Microsorum diversifolium* and *Pteridium esculentum*. The following is the distribution of recorded times for sporophyte appearance in the 106 samples that were positive: less than 60 days: 6; 61-120 days, 55; 121-180 days, 21; 181-240 days, 5; 181-240 days, 5; 241-300 days, 7 and more than 301 days, 7. There was a clear peak of the distribution in the period 61-120 days when over one-half of the spore samples had produced sporophytes. The median time for appearance of sporophytes was 106 days.

With some of the prothallus cultures that seemed to have 'become stuck' in their development, it was beneficial to transplant and thin them out onto fresh sterile soil in a new container. This allowed production of sporophytes, particularly from some of the New Zealand samples such as *Pyrrosia eleagnifolia* (an epiphyte) and *Blechnum fraseri* (a miniature treefern) that had not yielded sporophytes in the one-year-old primary cultures. These latter when kept in dead storage continued to remain stuck at the prothallus stage.

Sequels

A problem not so far mentioned is that of identity (Col. 6 of the Table). It can be somewhat frustrating to go to the trouble of spending 1-2 years growing a batch of ferns from a sample of spores and then find out that they are not what you expected. Until a sporophyte reaches a certain size, it may be difficult to tell whether it is what you thought it was or a 'fern weed'. The problem is particularly acute with ferns that are new to your experience, as most spore–grown ferns are likely to be. With wild-collected samples from ferny localities, the ripe frond collected may be contaminated with wind-blown spores from other species. I found this a particular problem in Australia and New Zealand where the forests contained treeferns that shed vast numbers of spores on the smaller ferns that were growing on them epiphytically and on the forest floor. As a result, I spent considerable time and effort transplanting and nursing along some unwanted visitors. I have seen it recommended that fronds should be washed before collecting the spores but this may not be feasible under expedition conditions. The entry 'OK' in the Table means no more than (that so far as I can tell) the fern is what I think it. Likewise the entry 'No' means I am pretty sure that what grew was a weed, while '?' means the sporophytes have not developed sufficiently at time of this writing, or are too small to be distinctive.

Of course, a weed may be an interesting and attractive specimen in its own right and, especially if it is from an exotic locality, well worth keeping. This is the case with the fern I grew from spores labelled *Mohria caffrorum*, a South African species where I have 'No' in the Identity column. The spores gave only one prothallus, which in turn yielded one sporophyte, possibly a *Cheilanthes* species.

A further source of disappointment is where the supplier of the spores either made a labelling error or had misidentified the source plant. This misidentification may also occur with mature ferns from commercial sources and one may not find out about it until an expert in that genus visits the garden. I have found this to be particularly a problem with *Asplenium*, *Dryopteris* and *Polystichum*, which are worldwide genera with numerous species, some with very subtle differences. In my experience there are very few fern experts who can confidently put a name to some Polystichums if there is no additional information on where the plants came from.

But I do not want to end on other than a very positive note. I have found the growing of ferns from spores an immense source of fun and satisfaction, and full of surprizes. It seems almost magical to transform specks of brown dust into beautiful green plants with decorative foliage and whose natural habitat is on the other side of the World. Since the shipment of spores between countries is not (so far!) regulated with the same strictness as living plants, there are possibilities of getting ferns that otherwise would be totally inaccessible. For the future, I plan to continue with growing fern species from the temperate regions of the World. I have hardly anything from South America, and only a small part of the fern flora of most other regions except the British Isles, Continental Europe and North America. In a forthcoming HFF Newsletter I hope to report on Canadian Ferns in a Scottish Garden.

I also have started to put extra thought and effort into growing spores from such reportedly 'difficult' genera as *Gleichenia, Grammitis, Hymenophyllum, Sticherus* and *Trichomanes.* Alertness, imagination and patience will be major requirements. Perhaps special growth media will be needed, or mycorrhizal inoculation. Also I shall be doing some literature searches to find out what is known about growing the species of these genera from spores.

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Table. Sources and ages of spore samples, and times recorded for appearance of prothalli and sporophytes. (NR, Not recorded).

Col. 1 Spore sample	Col. 2 Source of spores	Col. 3 Age of spores (yr)	Col. 4 Days to Prothalli	Col. 5 Days to Sporophytes	Col. 6 Identity
Actinopteris semi-flagellata	AFS (97)	3	25	80	OK
Adiantum cunninghamii	NZ (96)	0.25	56	277	OK
Adiantum pedatum aleuticum	PD (95)	0.25	19	89	OK
Adiantum reniforme	BPS (96)	1	38	420	OK
Adiantum reniforme	BPS (97)	1	11	47	OK
Anarthropteris lanceolata	NZ (96)	0.25	21	84	OK
Asplenium aethiopicum	PD (97)	0.5	38	206	OK
Asplenium bulbiferum	NZ (96)	0.25	NR	ca. 400	?
Asplenium daucifolium	PD (96)	0.25	47	271	OK
Asplenium flaccidium	NZ (96)	0.25	56	199	OK
Asplenium forezienze	BPS (95)	1	18	79	OK
Asplenium hemionitis	BPS (96)	2	39	127	OK
Asplenium lyallii	NZ (96)	0.25	56	179	OK
Asplenium oblongifolium	NZ (96)	0.25	39	133	OK
Asplenium obtusatum	NZ (96)	0.25	NR	179	OK
Asplenium platyneuron	AFS (97)	0.5	25	123	OK
Asplenium polyodon	NZ (96)	0.25	56	233	OK
Asplenium obliquum	AFS (97)	5	59	>130	?
Asplenium richardii	NZ (96)	0.25	52	294	OK
Asplenium saggitatum	BPS (96)	1	56	142	OK
Asplenium scleroprium	AFS (97)	0.5	25	106	OK
Athyrium pycnocarpon	AFS (97)	0.5	25	123	OK
Athyrium thelypteroides	BPS (95)	1	33	87	OK
Athyrium thelypteroides	AFS (97)	4	25	124	OK
Blechnum nipponicum	PD (97)	0.5	38	>212	?
Blechnum chambersii	NZ (96)	0.25	56	277	OK
Blechnum filiforme	NZ (96)	0.25	52	ca. 450	OK
Blechnum fraseri	NZ (96)	0.25	52	ca. 450	OK
Blechnum minus	BPS (95)	2	33	146	OK
Blechnum sp. (Alpine Chile)	BPS (97)	2	17	114	OK
Blechnum vulcanicum	BPS (95)	1	12	79	OK
Blechnum wattsii	NZ (96)	0.25	39	147	OK
Cheilanthes feeii	PD (95)	0.5	17	108	OK
Cheilanthes ineaqualis	PD (97)	0.5	17	108	OK
Cheilanthes ineaqualis	PD (97)	0.5	25	66	OK
Cheilanthes hirta	PD (97)	0.5	17	57	OK
Cheilanthes sp. (South Africa)	PD (97)	0.5	25	150	OK
Cibotium schiedii	PD (96)	0.25	17	101	OK
Coniogramme japonica	BPS (95)	1	12	321	OK
Cosentinia vellea	BPS (95)	1	12	41	OK
Culcita dubia	BPS (95)	1	39	81	OK
Cyathea australis	BPS (95)	1	13	88	OK
Cyathea colensoi	BPS (97)	1	47	212	OK
Cyathea cooperi	BPS (95)	1	13	85	OK
Cyathea cooperi Brentwood	BPS (97)	2	11	91	OK

Col. 1 Spore sample	Col. 2 Source of spores	Col. 3 Age of spores (yr)	Col. 4 Days to Prothalli	Col. 5 Days to Sporophytes	Col. 6 Identity
Cyathea cunninghamii	NZ (96)	0.25	56	96	OK
Cyathea dregei	BPS (95)	1	33	134	OK
Cyrtomium lonchitoides	BPS (95)	2	33	60	OK
Cystopteris alpina Regia	AFS (97)	2	11	106	OK
Cystopteris sudetica	AFS (97)	0.5	11	80	OK
Davallia tasmanii	BPS (96)	NR	39	108	OK
Dicksonia lanata	BPS (95)	1	33	97	?
Didymoclaena truncatula	BPS (97)	2	11	74	?
Diplazium australe	NZ (96)	0.25	52	139	OK
Doodia media	NZ (96)	0.25	56	81	OK
Dryopteris arguta	BPS (95)	1	13	113	OK
Dryopteris arguta	AFS (97)	0.5	25	123	OK
Dryopteris athamantica	PD (97)	0.5	45	>206	?
Dryopteris azorica	BPS (97)	1	17	96	OK
Dryopteris dracomontana	PD (97)	0.5	38	140	OK
Dryopteris dracomontana	PD (97)	0.5	21	96	OK
Dryopteris magellanica	BPS (97)	2	17	91	OK
Dryopteris sp. (Tenerife)	PD (96)	0.25	13	74	OK
Elaphoglossum apodum	AFS (97)	4	64	>130	?
Gleichenia microphylla	BPS (96)	1	58	96	OK
Gleichenia microphylla	AFS (97)	2	25	106	OK
Gymnopteris vestita	BPS (97)	1	11	270	OK
Hemionitis arifolia	BPS (97)	1	13	74	OK
Histiopteris incisa	NZ (96)	0.25	56	81	OK
Humata tyermanii	BPS (97)	2	28	96	OK
Hypolepis dicksonioides	NZ (96)	0.25	58	108	OK
Lygodium japonicum	BPS (97)	2	23	64	OK
Macrothelypteris torresiana	BPS (97)	1	13	64	OK
Microsorum diversifolium	BPS (95)	2	13	47	OK
Microsorum scolopendria	NZ (96)	0.25	69	331	No
Mohria caffrorum	BPS (97)	1	32	64	No
Nephrolepis cordifolia	PD (95)	0.25	17	NR	OK
Nephrolepis sp. (NZ native)	NZ (96)	0.25	69	160	OK
Notholaena marantae	BPS (96)	1	39	96	OK
Oleandra sp. (China)	AFS (97)	2	25	123	OK
Paesia scaberula	NZ (96)	0.25	39	92	OK
Paraceterach reynoldsii	BPS (97)	1	18	101	OK
Pellaea andromedifolia	BPS (95)	2	18	NR	OK
Pellaea glabella	PD (95)	0.25	17	74	OK
Pellaea quadripinnata	PD (97)	0.5	38	108	OK
Pellaea rotundifolia	NZ (96)	0.25	57	112	OK
Pityrogramma calomelanos	AFS (97)	NR	25	92	OK
Pityrogramma triangularis	BPS (97)	1	21	134	OK
Platycerium superbum	BPS (97)	1	18	105	OK
Pneumatopteris pennigera	NZ (96)	0.25	56	216	OK

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Col. 1 Spore sample	Col. 2 Source of spores	Col. 3 Age of spores (yr)	Col. 4 Days to Prothalli	Col. 5 Days to Sporophytes	Col. 6 Identity
Polystichum australiense	NZ (96)	0.25	39	290	OK
Polystichum lemmonii	BPS (96)	1	NR	108	OK
Polystichum vestitum	NZ (96)	0.25	56	275	OK
Polystichum sp. (South Africa)	PD (97)	0.5	38	174	OK
Pteridium esculentum	NZ (96)	0.25	32	56	OK
Pteris cretica	NZ (96)	0.25	38	96	OK
Pteris macilenta	NZ (96)	0.25	56	118	OK
Pteris tremula	NZ (96)	0.25	56	81	OK
Pyrrosia eleagnifolia	NZ (96)	0.25	52	ca. 400	OK
Stegnogramma pozoi	AFS (97)	3	NR	106	?
Sticherus tener	AFS (97)	0.5	25	75	No
Sticherus cunninghamii	NZ (96)	0.25	39	127	OK
Thelypteris hexagonoptera	BPS (96)	1	56	died	
Thelypteris hexagonoptera	AFS (97)	0.5	27	106	OK
Thelypteris noveboracensis	AFS (97)	4	25	97	OK
Thelypteris simulata	AFS (97)	3	27	135	OK
Woodsia alpina	BPS (96)	1	39	NR	No
Woodsia fragilis	AFS (97)	0.5	25	94	OK
Woodsia plummerae	BPS (95)	2	13	66	OK

A Method for Growing Ferns from Spore

Robert Louis Muller - e-mail : roblfern@esslink.com

I. PREPARE AHEAD:

a. Using a soil such as Pro-Mix, obtainable from a good nursery, moisten some with distilled water, and place in a covered baking dish.

b. Using a microwave oven, bake soil for 5 minutes at full power. Make certain that the soil has enough moisture (this is the secret to killing sphagnum moss spore so that it will not contaminate the fern spore growth).

c. Allow soil to cool, then fill the 8 oz. plastic cups with the soil to 1/2 Inch of the top.

d. Spray lightly with RD-20 solution mix as mentioned below, using a clean spray bottle, and then cover each cup immedlately with a Saran Wrap cover.

e. Theses containers are ready for planting.

Note: I always keep a few prepared cups on hand for the time of ripening fern spore. Mold spore could grow on this medium and ruin the planting. Thus, the use of the RD-20 mix and the Immedlate covering of the cups.

II. MATERIALS:

1. 8 oz. clear plastic cups
2. One gallon of distilled water
3. A hand spray bottle
4. Saran or freezer wrap
5. Physan (RD-20 solution comes as a liquid concentrate) continue reading for a source. The RD-20 solution mix is 1/8 tsp RD-20 concentrate to 8 cups of distilled water.
6. Ripe fern spore collected and dried in a mailing envelope.

III. PROCEDURE

1. Watch for ripening of the fern spore.
   a. Spore is ripe when it appears a chocolate brown color (in most varieties).
   b. Collect a single fertile frond and place in a mailing envelope and seal the same.
   c. Mark envelope with the name of the fern, date and location of the collection site.
   d. Allow envelope to stand for a few days, so that the spore is released in the bottom of the envelope.

Note: This process may be helped by gently tapping the envelope several times during the drying period.

2. Cut a small hole in the corner of the envelope.
   a. Remove the saran wrap cover from one of the prepared planting cups.
   b. With the open corner of the envelope pointing down directly over the soil, very gently tap the envelope. Run the spores through micron filters to remove the chaff. The spores will pass through to the soil.
   c. Spray with the RD-20 solution mix and immediately reseal with the saran wrap cover.
3. Place container in good light, no sun and in a location where the temperature will stay between 60 to 85 F.
4. In three to four months, possibly sooner, the first stage plants called gametophytes appear.
   a. They begin as a green fuzz on the surface of the medium and then proceed to a small fingernail size plant laying flat to the soil surface and often appearing heart shaped.
5. Once this first stage has been reached
   a. Remove the saran cover on each container
   b. Spray lightly with RD-20 solution mix, remembering to reseal quickly again.

Note: This action causes the fern to begin the second stage of its growth called the sporophyte stage.

6. Spray lightly again in 6 months if second stage plants (true fern leaves) are not showing. Be patient, different species of fern take different times to reach their stages.
7. If the spore was ripe and you see no mold growing on the soil, this indicates that everything should be all right.

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A Method for Growing Ferns from Spore *continued from page 17*

8. Little ferns must be 'hardened off' before removing to the pots filled with fresh pro-mix soil.
   a. 'Hardening off' - This is done by gradually removing the Saran Wrap cover on the plastic cup over a two-week period.
   b. This requires checking daily for the need to water the baby ferns. They will begin to dry, when the air hits them.
   c. Grow plants in the cup for a couple of weeks after 'hardening off' and before transplanting.

IV. PLANTING OUT

A. Plant up small ferns in fresh pro-mix in 1/4 inch clumps, a few clumps to a pot.

B. All pots used from this point on must have drainage holes in them. Do not try to separate individual little plants.

C. After transplanting, watch the watering and remember that ferns are not plants that like a lot of fertilizer.

D. Water when the top of the soil is dry to the touch and fertilize sparingly.

E. The use of a humidity tray is a good idea when growing these plants.

F. Spraying the tops of ferns even several times a day is not effective. I would also suggest using only 1/4 strength of the recommended amount of fertilizer and only once a month.

G. When watering, always allow ten times the water necessary to wet the soil, flow through the pot. This is known as 'leaching the soil'. This procedure will insure that no build-up mineral salts or excess fertilizer will remain, which could kill the fern's sensitive roots.

This method of growing plants from spore will work on local and tropical ferns alike. It looks harder than it really is, and once learned, will afford you with many years of enjoyment. Have fun with this most rewarding of hobbies, the growing of ferns from spore.

Good luck and good growing.

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Torrington, CT 06790-5144
(806)482-6257

RD-20 is (PHYSAN) which is a liquid concentrate greenhouse disinfectant for orchids. Most orchid supply houses would carry this product. This is where I purchased my supply.

Tropical Plant Products
1715 Silver Star Rd.
P.O. Box 547754
Orlando, FL 332854

"Yet Another Method of Growing Ferns from Spores"

James R. Horrocks - Salt Lake City

Those of us who grow ferns from spores more than likely have our own preferred methods. Some are relatively complicated and rather involved while others are more simplified. I have personally tried a number of methods - the inverted pot method, the peat-pellet method, the porous brick, even water culture. I have, however, in the last few years, settled on a method that is simple, and most importantly, proven successful. I have had bumper crops, even with hard to grow species.

First of all, I use a plastic container with a transparent lid that allows light through. Cool whip containers work very well. After some small pieces of charcoal are placed in the bottom, I add Scott's African Violet potting soil to a depth of an inch and a half after being firmed down. Then I carefully add distilled water to which a soluble fertilizer has been added at half-strength. Spoon-It, Rapid-Gro, and Miracle-Grow have all been used with good results. I use a large irrigating syringe to add the water. A turkey-baster can also be used. The soil is dampened but not made soggy. Finally the spores are added, carefully distributing them as evenly as possible. The container is then sealed with the close-fitting lid and placed under ordinary fluorescent lights.

When the first tiny fronds appear, they can be picked out and placed in small pots with African Violet potting soil watered again with the distilled water - fertilizer solution. These are of course placed in a humidity chamber of some kind and grown until they are a size that will ensure their survival. This method has been very successful, more so than anything else I've tried. The tiny sporelings are just fine if you don't get around to potting them up right away. I am presently experimenting with using plastic sandwich bags as containers instead of pots and so far the results look very promising.

One final note, for those of you who live in areas of less humidity and infrequent rainfall, as I do, there is a successful way of making the transition from the humidity chamber to the outdoor garden. It is done simply by adding one extra step - and that is: growing them in a shaded cold-frame for one season, beginning in early spring. By late fall, most of the new crop are large enough to be set out in the garden when the days are cooler. I usually do it on a rainy day. Those I have planted in this way have come up the next spring as if they had always been growing there.

For all my efforts, I am rewarded with healthy plants off to a good start, increasing each year in beauty and charm.

Fern Propagation

Sue Mandeville, Springfield, OR

1. When I receive the spore, I make white plastic labels that I tape to the package of the spore. Then when I am ready to "plant" the spore I have the labels all done. After planting I tape the label to the top of my already sterilized containers one at a time so I do not get the labels and spore envelopes mixed up.

   I use 1 pound micro-waveable containers I get from the deli department of any grocery store. They sell them to me in bulk (25 per sleeve) for 25 cents a piece. I fill the container with about 1 to 2 inches of soil (just the commercial stuff I buy by the yard) and then microwave (sterilize) that on high for 5 or so minutes (lids too, that are set loosely on the containers). The one secret here is to have the soil moist enough going into the microwave or it will get too dry. It comes out steaming. I microwave cups of water while I am sterilizing the soil so that I have a good supply of sterile water to use as the need arises.

   Once the containers/soil cool down enough to use, I dust the soil with the spore and place the lid on. The "planted" containers are put on a sand/heat cable shelf with 12 hours of light from cool white fluorescent lights and left until the prothallia emerge or I am sure nothing is going to grow...this can take from 1-3 months.

   I check periodically and spray with sterile water if they appear to be drying out.

2. Once the prothallia are big enough to move, I transplant them into a hort-tray that has a mix of sorta sterile soil (as above) with some leaf compost added. I say sorta sterile, because microwaving large amounts of loose soil is difficult and I am quite certain the soil, although it gets hot, is not sterilized.....at this stage I am not that concerned with complete sterility, and so expect to have a few weeds sprout....I've never been disappointed yet. On occasion some fungus starts up. When that happens I spray the infested area with some isopropyl alcohol, and this keeps the fungus from spreading....I may have to spray several times, but eventually it works to kill the fungus and of course any prothallia that were there, but it saves the entire tray so it is worth it.

Figure 1. Photo by Sue Mandeville

Another thing I have found is that unless the fern is one that is very rare or special I only transplant 10-15 prothallia bunches. I learned this restraint

Figure 2.

Photo by Sue Mandeville

only after many years of transplanting too many of any one thing, to the point I ran out of room, materials, and patience to get the job done at all.

3. I found the most wonderful clear plastic covers at a Portland Greenhouse supply store. They are over 8 inches tall and provide lots of room for growing ferns.

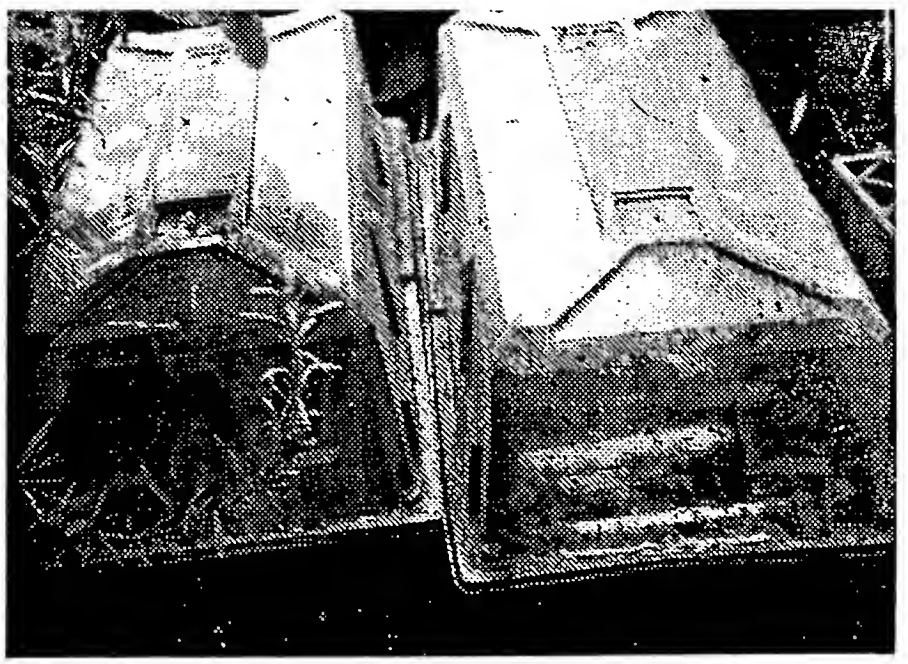
   The prothallia do not mature at the same rate so I transplant those that are producing mature fern leaves and leave the remaining prothallia in place. I fill the hole where I removed the maturing ferns with soil so to keep the moisture even. Once the ferns are big enough, I transplant them up to a two inch pot.

4. The final stage for me is to plant the maturing ferns in 6 inch pots and line them out on some steps that face northeast in the backyard. Some get donated to plant sales, some get given to friends and others are earmarked for my own beds....if and when I ever get them done!

Comments regarding starting ferns from spore: It is exciting business, because I often get a mixture of ferns from the spore packages. (I'm not complaining!) Once when I tried to start some terrestrial orchid seed using the same method as starting spore, I got bunches of ferns to "sprout", but no orchids. Makes me wonder what ferns I might get if I planted the "chaff" from overseas seed shipments I receive. Fern mysterys!!

Figure 3.

Photo by Sue Mandeville



Growing Fern Sporelings on Pumice Rocks

Jim Baggett - Corvallis, OR

After having a lot of problems with Blue-green algae in plastic boxes used to raise fern sporelings, I decided in 1995 to try a more natural method by sowing spores on pumice rocks. This was based on the observation that many sporelings develop on moss covered rocks in moist areas of my garden. Pumice rocks are especially likely to have baby ferns, with or without a moss cover.

The pumice rocks were selected for size, shape, and texture. They ranged in size from 9 to 12 inches in length, from roughly globular to flattened, and with lots of cavities and porosity. They were hosed clean, then cooked in our oven at 350 F for around 35 minutes (or longer for the largest ones). They came out of the oven quite dry and required considerable wetting to regain an acceptable amount of moisture, as determined by their weight. An alternative method of sterilization tried was soaking them in a household bleach solution followed by washing and aeration. Generally, spores did not germinate on the bleach treated rocks and one of them remained totally barren for a year before being returned to my rock pile. Another rock was hosed off and used without any sterilization. The results with this unsterilized rock differed only in the earlier development of a moss cover.

Spores were dusted over the moistened rocks in September and November, 1995. I usually overplanted, as I always do when using plastic containers, but time would demonstrate that in some cases I apparently did not have enough viable spores to get a crop.

The planted rocks were placed just under the south bench of my unheated plastic greenhouse where there was no direct sunlight. They were kept moist and occasionally fed with a complete soluble fertilizer (Rapid Grow) at about 1/2 t. per gallon of water. Moss and liverworts eventually invaded the rocks, but blue-green algae was not a problem, even though this pest is common in my greenhouse. When blue-green algae started to develop on the most exposed edge of one rock, moving it farther back into the shade seemed to stop the problem.

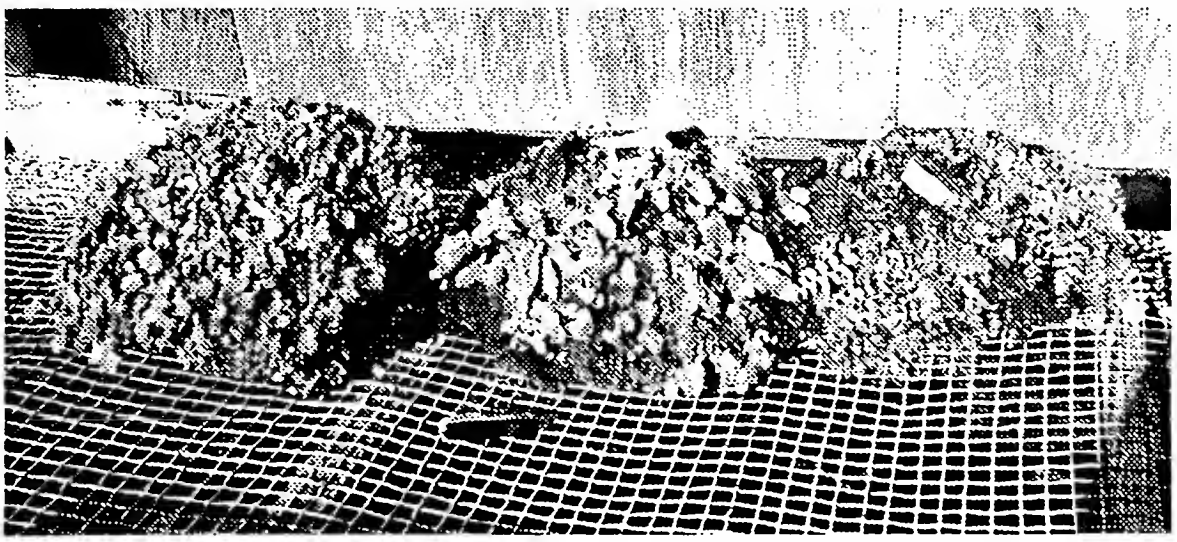
Prothallia developed abundantly from the spores of three fern varieties. A small form of *Polystichum munitum*, a *Polystichum setiferum* variety of questionable identity but of the *latipes* type, and *Phyllitis scolopendrium* var. *laceratum*. However, the *P. munitum* prothallia did not produce sporophytes and eventually disappeared in the moss cover. The *Phyllitis* produced a dense population of laceratum type plants which are healthy and about 1 to 2 inches wide at this time (October, 1997). About 75 of the *P. setiferum* var. *latipes* (?) plants were removed from the rock in late 1996, grown for a few months in plastic boxes, and are now healthy plants up

to 6 or 8 inches tall in 4-inch pots. There are probably 50 plants remaining on the original rock and doing well.

In addition to the three varieties described above, only about four identifiable plants resulted from planted spores of *P. setiferum* 'Follosum Grand Walton'. In this case, the spore crop used was of questionable quality. Also, three rocks planted with *P. polyblepharum* produced no plants of this species, but here again, the spores were of questionable maturity and quality.

The most conspicuous result from the experiment was the great production of self-sown plants from mature ferns living in the greenhouse. The first sporophytes to appear (by many weeks) were self-sown dwarf and other maidenhairs (*Adiantum* varieties). Then, wild type *Phyllitis scolopendrium* plants completely covered several of the rocks and now have leaves up to six inches long. The maidenhairs are mostly covered by the harts tongues. The *P. setiferum* var. *latipes* (?) crop was never threatened by self-sown plants, presumably because the planted ferns covered the rock before foreign sporelings could get established.

So, what conclusions can I make from this experiment? It certainly is feasible to raise ferns by this method if one has a suitable place to keep the rocks for a year or two. In my experience, the method is somewhat slow, but the plants I removed from the rocks were larger and easier to handle than the very small and crowded ones I have grown in plastic boxes. It seems apparent that some ferns may not be adapted to this method.



Ferns sown on pumice. Photo by Jim Baggett.

Growing Spores on Inorganic Media

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When first starting on my own personal fascination with ferns the best way for me to be able to experience many of these plants was to grow them from spores. Many were native to upstate New York but there were many that could only be found listed on the spore exchange of the American Fern Society. After ordering as many hard-to-find species and cultivars as I could, I traveled around collecting more for myself and to send in to the exchange.

Fortunately for me I worked in a greenhouse where I was permitted to experiment with the various techniques of spore germination. I thought that it would be so easy because the greenhouse had such excellent growing conditions. Soon I had thousands of small sporelings growing quite happily in small 2.5" pots or on peat pellets. My pride was soon replaced by irritation when I realized that many of the cultures were actually moss or algae cultures. On the cultures that did seem to sprout lots of gametophytes it was not long before algae or moss smothered them too. Worse, fungus gnats or shore flies laid eggs and soon my cultures were swarming with little larvae that ate up and tunneled their way through the last of my last good cultures. These difficulties were only moderately reduced when I sterilized everything and sealed things inside of plastic bags.

My problems were clearly related to the environment where I was trying to grow my spores and the techniques I was using. The greenhouse was very old and spores of algae and moss abounded in the air and were an element that could not be avoided. Even "clean" areas such as the headhouse proved to be just as problematic. I remember several times while I was sowing my spores that fungus gnats would immediately start buzzing around my fresh pots of sterile mix and land right in the middle of what I was doing. Then just to spite me they would try to fly up my nose or into my eyes!

To avoid the problems with insects I switched to an inorganic substrate to grow my spores. Next, to minimize contamination from moss or algae I sealed my cultures inside clear plastic boxes like the ones used to package alfalfa sprouts in the produce section. Last I moved the entire operation to my home and worked in my office. The two most effective substrates for growing spores turned out to be oasis* foam or rockwool**. With their high porosity and water holding capacity they seemed like the most ideal choices next to bricks. Besides it was always hard to find an old brick that had the right texture. Never mind trying to cut it or break into the right shape.

The following is a step by step guide to growing spores using this technique.

1. Obtain oasis floral foam or rockwool. Any color other than green is best so you can see the spores germinate. Cut material into cubes that will fit snugly in the bottom of the clear plastic container of your choice.

2. Soak, rinse and repeat three times to remove any surfactants used in the foam or rockwool. This also assures that the pH is not too far out of whack - it should be perfectly stable after rinsing.

3. Mix up one liter of half strength tomato-grow fertilizer. To this solution add 1/8 tsp. of the fungicide BANROT or CAPTAN.

4. Pour solution over oasis or rockwool cubes. Place cubes into culture vessels and cover them tightly with the lid. These containers can be stored for several months in this state until ready to use.

5. Place a small amount of cleaned spores on an index card. Gently tilt card so any debris roll off leaving the spores behind. If you are in doubt that there are any good spores in your sample, crumple it all up and sprinkle it over the media like oregano. Even though there will be fungal growth to start it will quickly run out of food by the time the spores germinate. *(Lygodium palmatum interestingly did not shed many spores for me even though I collected the spores in January. Extremely dense germination occurred right on the chaff one week after sowing.)*

6. Remove lid and invert card over the opening and give the card a tap to release the spores. Cover container as soon as possible. Work in an area not likely to have many algae or moss spores around.

7. Place two feet away from a double florescent fixture and try to maintain a temperature of 65 -75°F and stand back. A 12 hour photoperiod is enough but you could also have continual lighting.

Germination should be evident for most species after two weeks or it may take a lot longer for others if the spore is old. Contamination with algae and mosses can be effectively dealt with by dipping a q-tip in hydrogen peroxide and just dabbing the contaminants as soon as they appear (*usually in the first two weeks or longer if you keep taking the lids off to see what's happening*). They should be no bigger than a pinhead and must be stopped before they get out of control.

Nine to twelve weeks after germination the gametophytes should be ready to be flooded with the same mix of fungicide and fertilizer used to soak the media.

Sporophytes should be noticeable after 6 - 21 days if the gametophytes were mature at the time of flooding.

Sporelings are transplanted when at least two fronds have emerged. Transplant to a mix containing equal parts of sand, peat, perlite and vermiculite. Add dolomite (*not hydrated lime*) along with gypsum for calciophiles. A cup of each per bushel should do. Make sure that sporelings are kept in shade at this point and are gradually exposed to less humid conditions. Recycled soda bottles are great for out planting sporelings.

This method has worked so well that I have produced more sporelings than I know what to do with - just ask my gardening friends with new fern collections. This is also a technique that makes growing ferns from spores a more attainable goal for those who do not have the luxury of greenhouse space.

*Oasis can be found in almost any florist or garden shop and is used as the spongy block that flowers are arranged on.

**Rockwool is commonly found in stores or catalogs that cater to hydroponic enthusiasts.

A Systematic Method for Spore Germination

Reprint from the Hardy Fern Foundation Newsletter
Volume 5 Number 2, Spring 1995.

Samuel Tumey - Liberty, MS

I approach the subject of spore germination with some hesitation because there are any number of articles in print which make the process seem to be

a.) So easy that an idiot can do it; or

b.) So difficult that it should be considered more art than science and success attributed to beginner's luck.

I understand that many readers have developed a technique which works for them, and, as a result, will not be interested in further assistance. This article is for those who have not attempted to grow ferns from spores, who have not had success, or who are still seeking a satisfactory and convenient method. In the past twenty years since I was a graduate student (where I had wild-eyed ideas about looking for bioactive compounds from prothallia) I believe I have tried almost every published method except the use of sterile agar. Most methods worked to one degree or another. My justification for the method which I am about to describe is that it was developed expressly for the ordinary gardener with no knowledge of ferns. The initial concept was to produce and market a prepackaged kit which would allow the purchaser to follow a few simple directions and obtain a pot full of baby ferns. The kit was unmarketable for reasons unrelated to spore germination, but I believe my experience with spore cultivation for non-experts can be of some value.

Step 1: Collection and preparation of spores. The standard methods of spore preparation need not be reviewed in detail. I do not sterilize my spores, because on a liquid medium it is usually unnecessary. In the rare event where the spore leaf is extremely dirty, I prefer to wash the frond while it is still fresh rather than wash the spores. I clean my dried spore material by tapping it on a sheet of magazine paper. Most of the chaff will bounce off the end of the paper and most of the spores will stick to the paper. However, if you attempt to germinate the clean spore material and throw away the chaff, you may throw away the viable spores. Unless you know that your clean spore material will germinate, you should retain the discarded sporangial material for possible later use. In the case of *Dryopteris* in particular, the viable spores often remain attached to the sporangia and have to be germinated with the accompanying junk material. For safety's sake, shelf life of non-green spores should be considered to be slightly less than one year, although some may last longer. Better to germinate spores as soon as it is convenient to do so. Avoid contamination with other species where possible. Bear in mind that the common species got that way, that is to say common, by being able to dominate their habitat, and this includes inhibiting growth and reproduction of the prothallia of less vigorous species. *Theylypteris* is a common offender in this regard. One or two spores of some of the more vigorous species may be sufficient to substantially reduce the yield of a rare or valuable culture.

Step 2: Germination. I use a sterile solution of Hyponex Plant Food (7-6-19). Almost any fertilizer solution would probably serve as well. However, some fertilizer solutions are artificially colored. A colored solution would obviously be inconve-

nlent for viewing the prothallia in their early stages of germination. Concentration is of little or no importance, and I generally estimate the amount of fertilizer. I have germinated several lime- dependent species on this medium without adding calcium or adjusting the pH, and have had good success.

I use old-fashioned glass petri dishes, spared from the trash heap many years ago when labs went over to the plastic variety. Any oven-proof glass container will work just as well. (There are now available at your shopping mall miniature Pyrex or Corningware casserole dishes which are very similar to Petri dishes in size and shape.) Containers are filled with nutrient solution and sterilized by heating for 120 seconds in the microwave. (Before microwaves, I sterilized them in the oven.) Caution! This will be a superheated liquid and the dissolved material has already raised the boiling point of the solution considerably. Treat it with the respect which this implies!

Allow the solution to cool, then dust the spore material over the top of the solution. Rigorous sterile procedures, such as would be required in microbial cultures, are not essential as long as general rules of cleanliness are observed. The spores will float on top of the surface of the water held up by surface tension. The containers can even be moved around without any danger of sinking the spores. Cover the containers and place under artificial light. White light is fine, and special grow lights are not necessary. Depending on species, spores may germinate in one to four weeks. You may lift the cover and observe with a hand lens as you wish.

The young prothallia can be removed almost as soon as desired after germination. However, they will be easier to work with if removed before they form a thick mat. It is important to understand that the prothallia are now in a race with algae, which is also beginning to germinate on the liquid medium. At the first sign of discreet threads of algae, you must make plans to remove the prothallia to solid medium. The algae will not ordinarily harm the prothallia, but, if allowed to spread over the surface of the liquid medium, will cause inconvenience in removing and transplanting the prothallia. In rare cases, you may wish to remove prothallia to another container of clean nutrient solution, but this will allow a grace period of only about a week before algae again overgrows the medium. Better to transfer to solid medium the first time around.

Step 3: Transfer to solid medium.

Most people use soil for their solid medium. However, I consider my customized inorganic medium to be superior to sterilized soil. I use the proprietary term "Gorilla Gravel" for this medium.

TO MAKE ONE PIE PLATE FULL OF GORILLA GRAVEL

1. Use clean Pyrex Pie Plate ten inches in diameter.
2. Add 1/2 cup of Vermiculite.
3. Add 1/2 cup of commercial cat litter. Note: I borrow my cat litter from the bag before it goes into the litter box. I prefer the generic variety without the little green odor absorbing pellets, but have not noticed that they inhibit germination or growth.

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A Systematic Method for Spore Germination *continued from page 27*

4. Add 1/8 cup of Canary Gravel from the pet store or grocery.
5. Add 1/8 cup of Ground Charcoal from the local garden center or pet shop. Caution! Don't breathe the charcoal dust.
6. Mix all this thoroughly with a spatula.
7. Add 1/8 teaspoon of garden lime.
8. Add 1/8 teaspoon of powdered commercial fertilizer. Hyponex is fine.
9. Add 1/8 teaspoon of powdered Aluminum sulfate.
10. Mix thoroughly with a spatula.
11. Place pie plate in oven at 350 degrees for about one hour. At the end of the hour raise the oven temperature to 400 momentarily and then allow the oven to cool at its own rate before removing the pie plate. Note:If the household chef must have the oven, protracted cooling isn't absolutely essential. I just like to get my money's worth out of the cooking session in the hope that I can perhaps bake that last stubborn fungal spore to death.

You now have just about the best inorganic medium that money can buy. It holds water well without becoming gooey and buffers out at a pH of just under 7. I don't make any effort to buffer it up for lime-loving species. It has worked fine for *Adiantum* species which later required lime supplementation as adult sporophytes.

Now you need a container. It may be that any old container will do as long as it's clean and transmits light. However, I have two observations on this point. First, plastic is better than glass. The reasons are not entirely clear. I believe it has to do with the fact that polymer sheets are porous at the molecular level. Remember: Your cola will go flat in a plastic bottle, but it used to keep its fizz indefinitely in the old glass ones. I believe that one of two factors are at work. Either gas transfer to and from the container favors green plants over molds, or micro currents are created which prevent the fungal spores from settling. A third possibility is that organic polymers are better insulators. Glass is cold and clammy to the touch while plastic is not. This would have an effect on the condensation cycle and may be a factor in mold growth. I am unaware of this subject having been addressed in the literature on spore germination.

Better results are also obtained if provision is made for diffusion and circulation of air through the medium itself. This can be accomplished by exposing a portion of the medium to the air, i.e. an inverted jar over a dish which is slightly wider than the jar. (Think of using an inverted petri dish, where the large part marked "Cover" is actually used for the bottom and the part marked "bottom" is actually the top and rests on the medium rather than forming a glass to glass seal.) Satisfactory results can be achieved by cutting out the middle portion of a soft drink bottle, placing solid medium in the

bottom part; then fitting the top and bottom portions together without the middle. When using this arrangement, a layer of perlite should be placed in the bottom of the bottle for drainage. For my growing containers I use little rubberized polystyrene trays that chemical laboratories use for weighing samples. These are available from chemical supply companies for about four cents apiece. I use one for the top and one for the bottom. The top half is inverted, and the two fit together to form an enclosed container. However, the lips where the top and bottom meet do not fit tightly, and air is thus allowed to pass in and out of the container, but solid particles, such as mold spores, will ordinarily bump into the side of the container rather than passing into the growth chamber. The containers can be washed and reused at least once before they disintegrate from exposure to light but I always use a brand new set if the spore culture is valuable. I bought a thousand of these little trays a few years ago for about forty dollars and expect them to last the rest of my life.

I place about a quarter inch of Gorilla Gravel in the bottom of a tray. Then I dip the prothallia out of the liquid medium with a plastic spoon and drop them at random onto the solid medium. (A metal spoon is awkward because the prothallia develop static charges and tend to run from the spoon.) Avoid the temptation to spread the prothallia too thickly because they will increase considerably in size.

The trays need to be watered about once a week. I use tap water which I have sterilized by heating for 120 seconds in the microwave. This keeps down contamination from fungi and algae. Ordinarily the prothallia need no additional encouragement to mate and produce baby ferns. I find that *Dryopteris* sometimes has to be exposed to genuine sunlight followed by a bath from a medicine dropper before fertilization will occur.

I sometimes transfer the young ferns directly to prepared beds in the woods, taking care to cover them with plastic drink bottles for the first year. If it is inconvenient to transplant outside, I transfer them to rectangular trays filled with a mixture of potting soil and sand. Even when transplanted inside, the young ferns need to be covered with drink bottles because either central heat or air conditioning will dry them out. I place a priority on moving my ferns outside into the soil as soon as possible, because, like a litter of puppies, they are cute when little but they become a pain to care for as they grow bigger.

I find that the above procedure is one that can be used with confidence. Of course, there is always the odd chance of fungal contamination or desiccation due to neglect. However, as a general rule, by sowing spores using the above procedures, it is possible to know with reasonable certainty that viable spores will yield a good culture. In the case of ferns collected in the wild, it has been my experience that enough spores to dust two plates, usually from only one spore-bearing frond, will provide more baby ferns than the grower will have the patience to transplant.

Vegetative Reproduction in Ferns*

Martin Rickard - Kyre Park, Tenbury Wells, Worcs. WR15 8RP ENGLAND

To the general gardener and specialist fern grower alike the vegetative reproduction of garden plants is of fundamental importance. It allows multiplication of stocks with a minimum of effort and, more significantly, since many garden varieties do not breed true, it allows the production of stocks almost certainly true to the character of the parent plant. Of course in the case of sterile plants normal sexual breeding is totally out of the question.

There are four ways of building up fern stocks vegetatively:

1. By division of mature plants
   a) plants with creeping rhizomes,
   b) plants with erect rhizomes.
2. By bulbils,
   a) normally occurring bulbils on leaf,
   b) artificially induced bulbils on leaf bases.
3. By apospory - addressed in Joan Gottlieb's article
4. By tissue culture - addressed in Serge Zimberoff's article

1. Reproduction by plant division

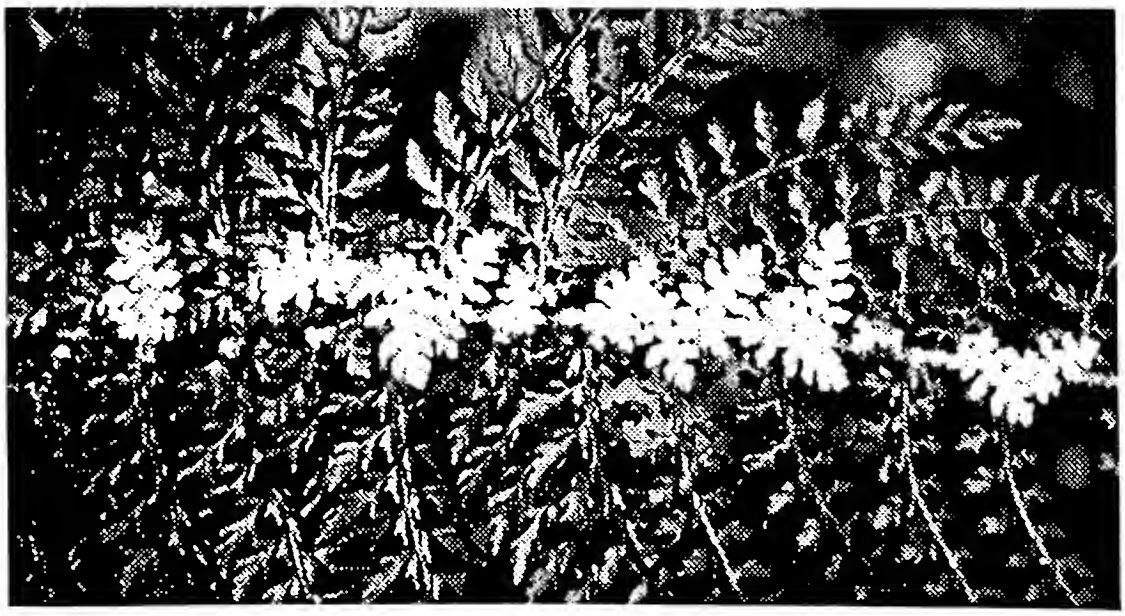
a) plants with creeping rhizomes

Division is straightforward; simply remove an inch or two of rhizome ensuring that there is at least one growing point on the selected piece. Obviously, disturb the roots as little as possible. It is best to use a sharp knife for this operation.

Examples of plants which may be propagated in this way are:

Adiantum x mairisii, A. venustum, Asplenium darioides, Blechnum fluviatile, B. penna-marina, Botrychium lunaria, Cystopteris montana, C. sudetica, Gymnocarpium dryopteris (including the plumose form), *G. robertianum, Lycopodium spp., Matteuccia struthiopteris, Onoclea sensibilis, Ophioglossum vulgatum, Phegopteris connectilis, P. hexagonoptera, Pilularia globulifera, Polypodium spp.* (all hardy species and hardy varieties; with some varieties of *P. vulgare* in the 'Cornubiense' section always choose a growing point in true character), *Thelypteris palustris, Trichomanes speciosum* (together with other British filmy ferns this is a special case needing to be grown in very high humidity).

The method to split a pot grown plant of *Adiantum spp.* is shown in photographs on pages 61 and 62 of *Ferns for Garden and Greenhouse* by Macself. He prises the



Polystichum setiferum bulbils. Photo by Sue Olsen.

*Reprinted with permission from Pteridologist Volume 1 Part 3 1986, British Pteridological Society

plant into small pieces using a small fork. This same system would apply equally well to border grown *Adiantums* as well as many other species.

b) plants with erect rhizomes

This includes the majority of ferns with the normal shuttlecock form of growth which produce side crowns. In some forms this can be a slow process with it perhaps taking several years to produce one offset. In others, side crowns can be produced so prolifically as to prevent the plant realizing its true potential, e.g. I have never seen *Polystichum setiferum* 'Pulcherrimum Moly's Green' in character unless it is kept as a single crown (even then only the odd frond or part of a frond has been truly pucherrimum!)

Examples of plants which can normally only be reproduced in this way are:

Polystichum setiferum

'Plumosum Bevis' -- some progeny from spores very rarely similar to parent, but probably not identical.

'Plumosum Grande Moly' -- very slow. 'Pulcherrimum Moly's Green'.

'Plumosum Green' -- once raised from 'Plumosum Bevis', can very rarely produce bulbils. In my opinion one of the most handsome of all garden ferns.

'Gracillimum' -- raised from 'Plumosum Bevis'.

Athyrium filix-femina

'Plumosum Druery'

'Plumosum Coke'. 'Plumosum Penny'. 'Clariissima Jones'.

Dryopteris filix-mas

'Bollandiae' -- the closest to a plumose fern yet discovered in *Dryopteris.*

'Ramosissima Wright' -- possibly a variety of *Dryopteris affinis.*

Several hybrids including:

Asplenium x alternifolium, Asplenium x costei, Asplenophyllitis microdon

All these varieties and hybrids are uncommon because the production of side crowns is usually a slow process. By a strange quirk of fate this list includes some of the very best fern varieties we still have in cultivation. Some of these may occasionally be available at specialist nurseries but quantities will always be limited. Those of us who have any must therefore do our best to distribute them among other enthusiasts whenever possible.

In *Ferns for Garden and Greenhouse*, page 66, Macself gives a full account of how to separate crowns. By reading this some useful tips may be gleaned, but I don't think any disastrous mistakes are likely if normal common sense is used.

Of course this technique is not restricted to only the rare treasures, but I should perhaps make two cautionary points -- firstly, take extra care with Scollies, they can often be difficult to split cleanly, and secondly beware of the problems of some *Asplenium*s, Reg Kaye (*Hardy Ferns,* p. 108) describes a disaster he had when trying to split the only plant of a form of *Asplenium trichomanes* 'Incisum' found by J.

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Vegetative Reproduction in Ferns *continued from page 31*

Barnes, after the division all pieces died and so therefore that was the end of that variety. Conversely, I find *Asplenium* hybrids split easily, e.g. *A. x alternifolium* and *A. x costei.*

2. Reproduction by bulbils

(a) bulbils occurring along the leaf rachis

Although relatively few ferns produce bulbils some good varieties can be propagated by this means. Simply lay the bulbiferous leaf onto soil; it may still be attached to the plant, or preferably detached and laid in a pan. It is important to ensure that the bulbils make good contact with the soil so that developing roots do not dry out. Equally it is important to ensure that the crown of the bulbils, and any young fronds, are not covered by soil as they need to receive light. If completely buried there is a good chance that rot will set in and kill the young plant. Examples of plants which can be propagated from bulbils are:--

Polystichum setiferum

'Iveryanum'

'Divisilobum Bland' -- bulbils sometimes difficult to strike.

many other 'Divisilobums', 'Acutilobums' and 'Multilobums' -- some forms only sparingly bulbiferous even when well grown.

'Plumoso-multilobum'

'Plumoso-divisilobum' -- bulbils only seem to be produced very sparingly, and even then only on well established plants; perhaps one bulbil every 2 or 3 years.

'Plumosum Green' -- very sparingly as above.

Polystichum proliferum and hybrids produced from it.

Asplenium flabellifolium -- from New Zealand
Asplenium tripteropus -- from Japan
Asplenium rhizophyllus -- from North America
Adiantum edgeworthii -- from Japan

} Like *P. proliferum* all these are bulbiferous towards the tip of the frond.

Asplenium scolopendrium -- bulbiferous leaves are very rare but have been reported once or twice, including quite recently.

Huperzia (Lycopodium) selago -- the upper parts of most stems bear little bulbils or gemmae, these readily fall from the plant and in suitable conditions will root and grow.

b) artificially induced bulbils on leaf bases

Asplenium scolopendrium and its varieties produce this type of bulbil. The bulbils are rarely, if ever, visible until appropriate steps are taken to aid their development. Curiously, this method seems to be immersed in a mystique which deters all but a few members from trying it. In reality it is a simple and quick way of building up stocks of scollies which could otherwise only be multiplied by splitting off side crowns. My system has been borrowed from many sources -- notably Mary Potts, I proceed as follows: --

1. Clean plant by removing as much soil as possible, as well as unnecessary roots.
2. Peel old leaves downwards so that they snap cleanly at their point of contact with the caudex. These leaves may be several years old and look dead, but

when separated they will be seen to be plump and green if only for the bottom 1/4 inch or so of their length.

3. When all leaf bases have been removed carefully, split the plant into crowns if desired or simply replant it as it is. It is unlikely that it will suffer any noticeable setback.
4. Remove the live basal section of each detached leaf to a maximum length of about one inch but probably less, wash it and plant it *upside down* in a pan of sterilized compost. By upside down, I mean plant so that the end that was attached to the caudex is sticking up out of the soil by 1/8 to 1/4 of an inch.
5. Keep the pan close by placing it under a clean sheet of polythene and misting with water as necessary. Close inspection will soon reveal the production of small green blisters around the tip above the soil -- sometimes as many as 10 per leaf base. Each of these will develop into a young fern given good husbandry.
6. Leave to develop into recognizable plants, perhaps 1/2 an inch high, before carefully teasing them off the leaf bases and pricking out in the normal way.

In summer this whole process might be completed in three months but over winter growth is, of course, much slower. As I write, I have about 250 bases neatly panned out from a single plant of *Asplenium scolopendrium* 'Crispum Bolton's Nobile' I lifted briefly in the autumn. Already I have more than 250 bulbils formed (after 3 winter months), but as yet no recognizable young plants. Previous experience tells me I will not get 250 mature plants from this batch, but I put that down to incompetence! There is no reason why a careful grower should not get almost 100% success.

It is reported that this technique also works on certain other species. William Cranfield (past President of our Society) *in British Fern Gazette* Vol 7, No. 12, p. 298, 1950 gives details of how this method can be used to raise *Oreopteris limbosperma, Athyrium filix-femina* and *Polystichum setiferum* as well as *Asplenium scolopendrium.* Apparently *Oreopteris* is easy to bud as with *A. scolopendrium.* With *A. filix-femina* a portion of the old caudex should be removed with the base. No specific tips for raising *P. setiferum* by this technique are given but it appears to be much more difficult.

Lady ferns can also rarely produce bulbils on their leaves. Reference to this in early issues of our *British Fern Gazette* have been pointed out to me by Ray Coughlin. In Vol. 1, p269 (1912) Druery mentions that the plumose lady fern *Athyrium filix-femina* 'Axminster' bears bulbils associated with the spore heaps, while in Vol. 3, p. 134 (1916) Druery confirms that this is true of most plumose lady ferns. More recently, Vic Newey has dramatically demonstrated the presence of bulbils on some of his lady ferns.

While, from the above, I hope it is clear that most ferns can be propagated vegetatively, it must be pointed out that in many situations vegetative reproduction is far from being the ideal way to raise ferns. The benefits of fern propagation sexually from spores are well shown by the range of new forms raised by some of the best growers. Also, the tendency to produce only one or a few daughter plants at a time has contributed to the great rarity of many of our best cultivars, e.g. *A. filix-femina* 'Clarissima', *A filix-femina* 'Victoriae' (original clone), etc. These ferns can only be reproduced true to character by the removal of side crowns -- which are, sadly, produced all too rarely.

Fern Propagation Using Tissue Culture

Serge Zimberoff

Santa Rosa Tropicals has used tissue culture for propagation since 1973. Whereas we are known for our work with ferns, we actually were the first to use these methods with Anthurium, Dieffenbachia and Spathiphyllum.

Some of the discussion that follows will seem 'generic', but generally it applies to ferns and other herbacious plants. In this article I hope to answer the following...when should tissue culture be used for propagation, what is tissue culture propagation and, briefly, how is it done?

Tissue culture cannot compete with the propagation of plants by standard means. If cuttings can be taken, divisions made easily and in large numbers, seed sown, or if viable spores are available then tissue culture is neither practical nor cost effective. Additionally, the chosen plant needs to be needed in large enough numbers to make it a practical choice. Only, if the above criteria are met, should tissue culture be used for propagation.

Ferns have an added paradox that makes it so much more interesting. Often a particular plant won't produce spore until it is quite mature. However it may be a tree fern or staghorn that has only one growing tip! Cutting out that tip will kill the plant and there is no guarantee that the tip will grow in culture. Waiting for spore may seem like forever. Ah yes, what to do? (Our secret is to not mention to the person that is doing the surgery that this is the only one of these ferns in the western hemisphere until after the deed is done!)

As far as the question, what is tissue culture, a rigorous description might look like this... Plant tissue culture is the sterile culture of plant tissue, such as cells, meristems, shoot tips, etc., in a vessel containing a microbe-free nutrient medium under environmental conditions suitable for plantlet development. (Whew...what a long sentence!) However, if you think of it as a highly developed life support system for extremely tiny cuttings you will actually have a better model to picture in your mind.

Early unsuccessful work with plant cell culture postulated that unidentified plant hormones were necessary for regulating cell division, growth, and differentiation. As plant hormones were discovered the way was opened for successful culture of plant tissue. The first active plant hormone to be identified was the auxin indoleacetic acid (IAA). The addition of coconut milk to plant tissue culture media was found to stimulate the growth and development of the cultures. Investigations directed at discovering the active principle in coconut milk resulted in the discovery of the first cytokinin, kinetin.

Work with tobacco callus found that organ development was controlled by the concentrations of auxin and cytokinin in the culture medium. High auxin levels induced root development and repressed shoot formation, while high cytokinin levels had the opposite effect. Subsequently it was shown that the auxin to cytokinin ratio controlling organogenesis is a general phenomenon.

The first extensive use of tissue culture for rapid propagation of ornamental plants was with meristem culture to free Cymbidium orchids of virus. Pathologists were interested in the elimination of the virus but readers of the reports noted the multiplication potential of the technique and so began the orchid tissue culture industry. (Compare orchids to the parameters noted above required for practical tissue culture propagation and you will see it is an exact fit!) Following on the heels of this work, others began working out the chemical and hormone requirements for other herbacious plants.

For all plants, ferns included, there are basically four separate stages to go through on the path from adult plant to adult plant. These descriptions were first used by your author (using rockets as the analogy for first stage etc.), and have since been almost universally adopted as the descriptive terms for these steps.

Stage one...explanting from the chosen parent plant. The major objective here is to establish a non-contaminated culture. This explant tissue is usually initiated with tissue from shoot tips, lateral buds, petiole or leaf sections. With ferns there is usually a choice of rhizome tips or growing crown tissue, depending on the growth habit of the plant. Additionally crowns of bulbils or pups provide good starting material.

The major problems at this point are in choosing the correct medium and then being able to kill the normal bacteria and fungi on the surface of the tissue without killing the explant. This is normally done with a 10% bleach solution with the times and means of exposure determined empirically.

Stage two...once tissue has developed sufficiently and is found to be contaminant free, it is transferred to this stage for multiplication. There is not always a clear cut step between stage one and stage three. With some plants multiplication may be incorporated in stage one and sometimes stage two may be the final stage with a formal stage three omitted.

Multiplication may involve formation of callus, from which adventitious organs or embryos develop, or stimulation of adventitious shoot growth. The first method allows a faster increase of plantlet initials but can lead to production of genetically aberrant plants, whereas plants developing from adventitious buds are typically less subject to genetic drift.

Stage three...if plantlets have not developed in stage two, where they are able to survive the transition into the big, bad world, then this stage is necessary. Typically plantlets or tissue is divided on a sterile surface using sterile instruments and this material is placed on a medium that has reduced levels or even no plant hormones present.

Stage four...this is where the plants are moved to soil. Anyone who has grown ferns from spore has a pretty good feel of how to manage this particular stage. The tiny plant is going from very high humidity and low light to the exact opposite. Care must be taken here to allow the humidity to fall gradually and the light levels to increase, but without reaching fatal leaf temperatures. This stage is often very diffi-

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Fern Propagation Using Tissue Culture ***continued from page 35***

cult because many plants grown in sterile culture do not produce the waxy cuticle covering over the surface of the leaves which helps to control water loss. New growth must be controlled so that new shoots have a chance to appear before the initial foliage is allowed to die back or the plant will surely die.

This is admittedly a bare bones overview of fern propagation by tissue culture. Those interested in further information would do well to look at PLANTS FROM TEST TUBES, by Lydiane Kyte. There is a fairly recent edition that goes into great detail.

Fernet (Formerly Pteridonet) Conversations and Observations on Fern Propagation

The following discussion on propagation was prompted by an article in Horticulture Magazine featuring Tony Avent. This issue also had an article on propagating ferns. For those of you not familiar with fernet it is an e-mail mail list. To subscribe send the command: Subscribe Fernet First Name Last Name to: macjordomo@koning.ecsu.ctstateu.edu.

Tony

The article on you & your business in the March Horticulture Magazine was wonderful!!>.....

>Those of you who do not get it; it is worth the effort. Also had an article on growing your own ferns from spores, etc. Would love to hear opinions (of those of you who have Propagated from Spores and have access to the article) on what you think of the article. Tony can you (& would you) comment on the article, for this amateur?

Good luck,
Mary Ann

>I haven't seen the article yet, so can't comment on that. We have many failures raising ferns from spores, but finally hit upon a seemingly foolproof method. Fill 4" pot with potting soil, then drench pot and soil with boiling water for sterilization. Dust spores on top of soil. Seal pot inside ziploc freezer bag, then wait. Extremely pleased with our sucess.

Tony Avent
Plant Delights Nursery
9241 Sauls Road
Raleigh, NC 27603
USA

>Just want to add a couple of points to Tony's posting. The boiling water on the medium goes a long way toward preventing contamination with molds and mosses. Not absolute, but the other methods that sterilize pots, media and spores as well as watering are more trouble than they are worth. Just make sure that there is no fertilizer in the media. The boiling water will put it all in solution at once. Don't need fertilizer to start spores anyway. Once they get past the gametophyte stage then you can always water with 1/4th strength of whatever fertilizer you use on the rest of your plants. Make sure that your set-up receives good light, but never direct sun. Without light, they won't grow. With sun, they cook! Nancy Swell

>To add a bit to Tony and Nancy's ideas, I've evolved a pretty simple way to sterilize the medium for spore growing. Pouring boiling water has the disadvantage of causing any perlite in the mix to float to the top. Microwaving is very thorough for sterilizing, but transferring the medium to containers is a bit self-defeating unless you use a sterile technique.

At the suggestion of my wife, I have been cutting off the bottom 4-5 inches of a gallon milk container, punching about 6 holes in the bottom, filling it half full with sopping wet mix (I use 50-50 Promix and Turface), covering the top with saran wrap and microwaving for 20-30 minutes. The plastic may bend slightly but doesn't melt in the microwave. The steam generated from the soggy mix does a first rate sterilization job, and as soon as the mixture has cooled, the spores are scattered and the container placed in a "Baggie" or Ziploc.

If the mixture has been wet enough to start with and the microwaving isn't prolonged, the amount of moisture is usually right, and the bag doesn't need to be opened for months.

I have seen very little contamination difficulties using this technique.

Jim McClements

>Hello Everybody!
Further to all the excellent advice given on the sterilizing of composts for spore sowings. To those who wish to use the microwave most of the larger supermarkets use plastic containers in their 'delis' that are microwave proof thereby avoiding the need to transfer the compost from one container to another. You can also purchase microwave clingfilm which can be used to wrap the container prior to treatment.

Matt Busby

>An even simpler way to start spores is to use peat pellets which are already sterile. I put two pellets into a 2 x 4 plastic box, or whatever I can find, pour almost boiling water over and wait until cool. Then I pour off the extra water and sow the spores. Put the lid on tightly and wait. I keep them on a north window sill where they get lots of light but no sun and the temperature is fairly constant. I've been doing this about 8 years and have never had a problem with moss or any other contaminates.

Jocelyn Horder

>Hi!

I don't know if this horse has been beaten dead yet, but what I do may seem funky, but it works. I use the jiffy sevens and put each in an aluminum foil cupcake liner and cover with a plastic cocktail glass. It makes a miniature little humid dome. Jiffys can be kept wet by putting water in the cupcake liners.

Shelley Dillard
Propagator
Morris Arboretum

>Don't you just hate people who say "Been there, done that"? My problem with the Jiffy-7's was the netting that held them together. Had a problem getting the sporelings free from that. For a while I cut the netting away from the top, but then that created another problem because they didn't have enough to hold them together. Somewhere along the line, I went back to using a peat-based potting mix, in a small flat. (The standard sflat 10.5 x 21 holds 12 of these small flats). I'll be honest with you, about as near as I come to sterile culture is that if there is any water

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left in the teapot, that is what I use. Unless the planting mix has been sitting around open, there is not all that much chance of contamination. I think that when it does happen that the infection has been carried on the spores. This is the only logical conclusion that I can come to when 11 out of the 12 are free from any contamination..... . Nancy

>Hi Nancy, I forgot to mention that after I have soaked the peat pellets I then tear the netting off and level the peat with a fork. Then I sow the spores. I think it is so easy this way and clean too.

Jocey

>Hello all you Ferners,

I'm new to pteridonet and have spent the last month just listening. Great stuff! All of my experience with ferns has been in the wilds of northern Minnesota. I haven't tried to grow ferns from spores but am anxious to try. Can anyone tell me how long your average *Athyrium, Onoclea,* and/or *Osmunda* spore are viable after the sporophore has been dried for herbarium mounting? Air dried, not using a dryer.

Thanks,
Dan - Botanist

>Dan,

The *Osmunda*, being green spore would not be viable at all. The *Onoclea* may be viable forever under almost any conditions. The *Athyriums* might vary, depending on the species. Probably for several years.... Probably also depends on storage conditions..... Nancy

>FYI, Jiffy also makes a product called the Jiffy-9 which is basically the same as the Jiffy-7 but slightly smaller diameter and no netting - they hold together pretty well unless handled often.

Clinton Morse - Greenhouse Manager

>I know that Jiffy makes a peat pellet without netting although I've never had a problem pulling the netting apart when I'm ready to plant my babies. Just rip away with your fingers. Also to respond to the people with sterilization problems, I use boiled/distilled water to soak my pellets in and if I have a problem later, I use a spray of either benomyl or banrot and it usually clears the problem right up.

Sharon Banister

>An interesting variation on the theme. What exactly are you referring to when you mention "protection fleece"? Will any light fabric (a bit of old sheet) do? I also think it is a good idea to try to grow the spores in a non sealed environment. Most of my failures have been in the hardening off stages.

>Peter Richardson wrote:

Can't resist boring you with my favoured variation on sowing fern spores in a pot. I three-quarters fill small shallow plastic plant pots with Levington (peat-based) seedling compost, from a

clean new bag, I don't usually sterilize but if I do I do it the microwave way. Then after the spores are sown, I cover the top of the pot with a piece of protection fleece, and hold it in place with a rubber band just below the rim.

Frank Skelton
Vancouver B.C.
Canada V6S 1C8

>Hi pteridonetters!

In the last issue of the American Fern Journal there is an article on extending the viability of *Equisetum hyemale* spores. Anyone interested in this group will surely find it interesting.

"Extending the viability of *Equisetum hyemale* spores" by Dean Whittier American Fern Journal Vol. 86, number 4, October-December 1996. page 114.

ED SALGADO

I read this paper this afternoon. Two methods that show promise are discussed, freezing dry to -70C, and immersing in glycerine and freezing to -10C. As we routinely store bacteria by a combination of suspending them in 30% glycerol and then flash-freezing them to -80C, it would be interesting to try this with green plant spores. If I've got time in the spring I will try it with *E.telmateia*. (An alternative is to keep the gametophytes going. My *Gleichenia microphylla* ones are four years old and still producing new sporophytes - in vitro).

Peter Richardson

>Dear Fern People

I am having a certain amount of success with raising spores on MS medium (quarter-strength and with charcoal) with no sugar or other organics in it apart from the agar, and sowing the spores straight from the packet. I started doing this because I had a number of sporelots which I deemed to be fragile, either because they are green (*Leptopteris superba*) or not properly mature, and imagined they would not stand being bleached. Spores sown like this germinate and grow very fast. In a growth room kept at 24C, I have had the first leaves of apogamous sporelings of *Pteridium esculentum* unfurled 33 days after sowing the spores, and the first leaves of apogamous *Adiantum hispidulum* sporelings after 45 days from sowing. So far, the gametophytes have a quite normal shape, and are not all ruffled and mossy as they generally are when grown with 10g/l sugar. Antheridia and archegonia do not appear as early as when sugar is present, though as I mention above, that doesn't hinder those that do it apogamously! Bacteria do not seem to be a problem in this system, but it is hit and miss whether you get fungi which can grow either with the agar or on the gametophytes and make a nuisance of themselves. Most of the fungi that do grow do so only weakly, and just have a few slender hyphae snaking around the gametophytes. However a few fungi rot the gametophytes, and I yank these out as soon as I see them. Green algae are also a nuisance that can swamp areas in green slime. If a culture looks as though it is afflicted with one of these problem microbes, I salvage some of the clean ones to a new vessel.

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As well as speed, I have got a greater range of sporelots to germinate in non sterile culture than on compost, particularly with tropical ferns (*Dicranopteris,Dipteris*). I have an *Adiantum* culture at home, and take the lid off (it is in a baby food jar with a Magenta Corp. plastic lid) to see how its doing with no detriment. This means that anyone could grow spores in-vitro at home, with just the medium in the vessel provided. However it helps a lot to have a binocular dissecting microscope on hand, to see what is going on (not a standard piece of household equipment, I admit)

Peter Richardson, Cambridge U.K.

>Peter,

What exactly is "MS medium (quarter-strength and with charcoal) with no sugar or other organics in it apart from the agar"?

Robin

>MS medium is a recipe for tissue culture media. This recipe was developed by Murashige and Skoog for the purpose of culturing tobacco and was published in 1962. It was found to be suitable for many other plants and so has become the most commonly used media. MS is available prepackaged from Sigma - to make it just follow the instructions on the packet. Ferns generally respond better to a 1/2 or 1/4 strength of the original recipe. Activated charcoal is generally added to absorb toxins and sometimes to reduce light to enhance root growth. Once sugar is added, this media and the plant material needs to be sterilised as bacteria will grow and multiply rapidly, so it would not be used in an unsterile environment.

Les Vulcz
Beech Forest Victoria

>Robin -
I thought you would ask. Here is the composition of Murashige & Skoog (1962) medium, omitting the organics (glycine, thiamine, nicotinic acid, casein hydrolysate etc.)

formula	final conc in medium
NH_4NO_3	1.65g/l
KNO_3	1.9/gl
KH_2PO_4	170mg/l
$CaCl_2$	440mg/l
$MgSO_4.7H_2O$	370mg/l
FeNaEDTA	36.7mg/l
$ZnSO_4$	8.6mg/l
$MnSO_4.4H_2O$	22.3mg/l
H_3BO_3	6.2mg/l
KI	0.83mg/l
$Na_2MoO_4.2H_2O$	0.25mg/l
$CuSO_4.6H_2O$	0.025mg/l
$CoCl_2.6H_2O$	0.025mg/l

This is a standard plant tissue culture medium that most plants will grow on. There are a wide range of other media devised for particular groups like specific orchid genera, the Ericaceae, or pines. We use MS for all our research on Eucalyptus, potato and tobacco at work, and ferns seem to do well on it. The only problem I have had is that young sporophytes of all of the Gleicheniaceae go yellow on it, the longer they stay on it. Assuming this is a manifestation of calcifugy(??) (well, calcium-hating, anyway) I have made a modification in which the calcium chloride concentration is reduced twenty-fold, and the Iron-EDTA concentration is kept at half-strength while the other nutrients are reduced to quarter strength. It's too soon to see if this is going to be better.

To play about like this, I make up each batch of medium from separate stock solutions for the major salts, (100x final conc.) and the seven micronutrients are bunched together in one stock solution.

I add 1g/l of activated charcoal to this, because it makes the ferns happier for reasons I don't know. It makes the medium black and opaque. The medium can be gelled with agar at 6-8g/l or Phytogel, (proprietory gellan gum) at 2g/l. pH should be 5-5.8, and the medium is autoclaved at 121C for 15mins. At home, this is conveniently done in a pressure cooker; 15 pounds per square inch for 15min.

I put 100ml medium in each culture box.

You can buy ready formulated MS media, as sachets to make up 1 or 5 litres, from Sigma. The one-litre sachets cost 1.40 UK pounds. They have expanded their range of plant media this year. Unfortunately private individuals cannot buy chemicals from a company like this, but a fern society might be able to. Sigma also supplies the individual chemicals used in this medium.

Probably the ideal container is the Magenta Corp.(Chicago) tissue culture box. It is square, transparent, autoclavable, has plenty of headroom (important not to let ethylene build up) and has a lid that allows gas-exchange with the air outside, without letting microbial spores in. It is also rather expensive. A cheaper alternative is to use glass baby food jars, for which Magenta sells a replacement lid with similar properties to their purpose-designed vessel. In the U.K., these can be all be bought from Sigma-Aldrich, but only in bulk quantities. There are probably loads of small jars that could be used, but the closure should not be airtight, but not too loose or the medium will dry out quickly. It is best if the lid is translucent.

U.S:
Sigma Chemical Company
P.O.box 14508
St. Louis, Missouri
1-800-521-8956

Canada:
Sigma-Aldrich Canada Ltd.
2149 Winston Park Drive
Oakville, Ontario L6H 6J8
800-565-1400

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U.K:
Sigma-Aldrich Company Ltd.
Fancy Road
Poole
Dorset BH12 4QH
0800 373731

Australia:
Sigma-Aldrich Ltd.
P.O.box 970
Castle Hill, NSW 2154
1-800-800-097

Peter Richardson Cambridge, U.K.

>I find the hardest part of growing ferns by micropropagation (using MS medium) is the hardening off process. The ferns are in a sterile environment and they have to be brought out of the container and introduced into the real world, to finally produce a fern that can be planted in the garden and thrive. Losses can be high if they are not getting my full attention. It is a lot easier to harden off ferns that have been propagated on compost/peat. To use an "unsterilised" tissue culture medium to sow spore on, I presume you would get the quick germination and rapid growth but the ferns would be hardier at hardening off time as they have already been exposed to the world. We are currently using a peat/ sand mix to propagate ferns on. Has anyone else tried germinating spores on MSmedium (unsterile), or other media?

Les Vulcz

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